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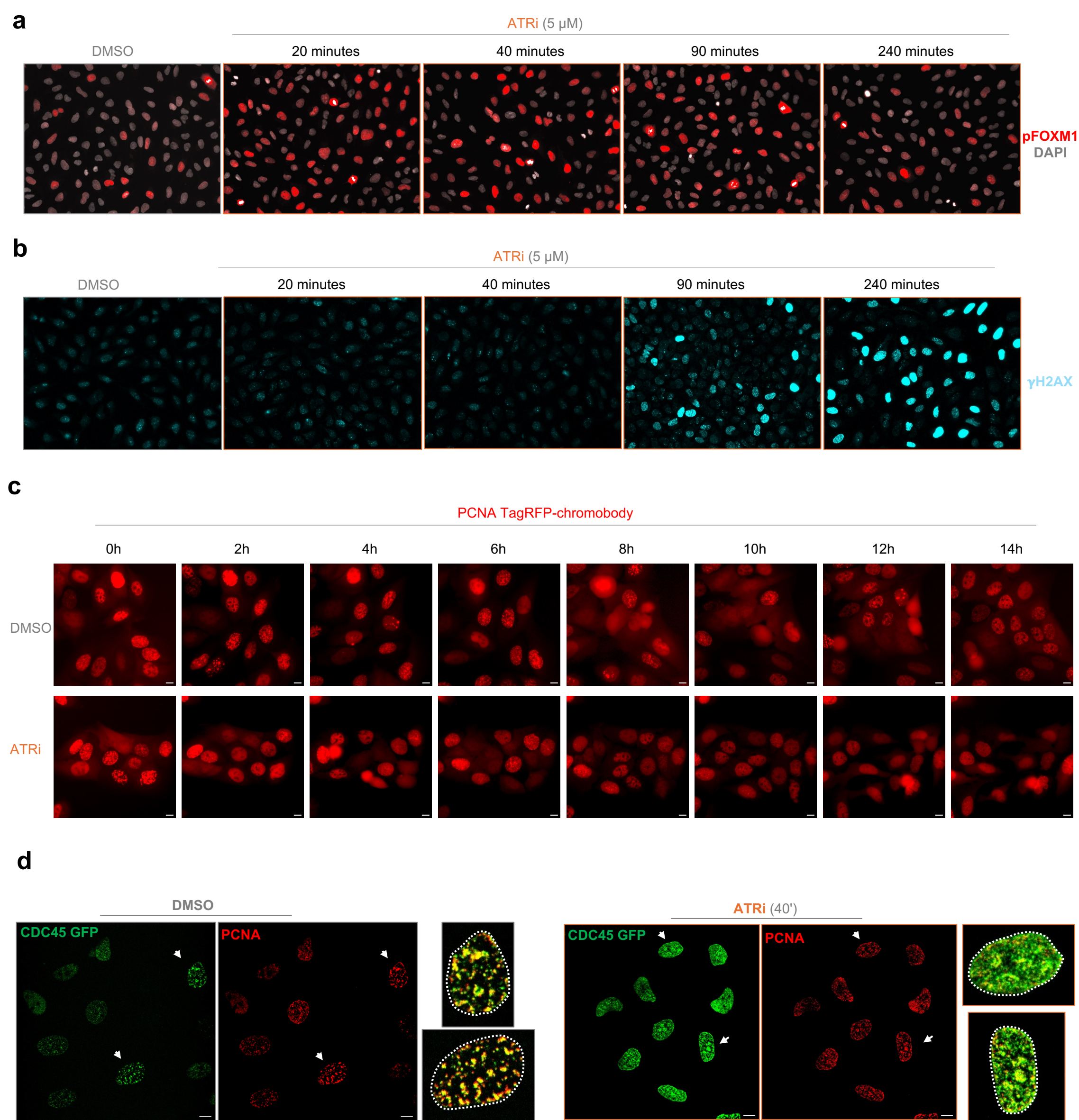
**Supplementary information**

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**PAF15–PCNA exhaustion governs the strand-specific control of DNA replication**

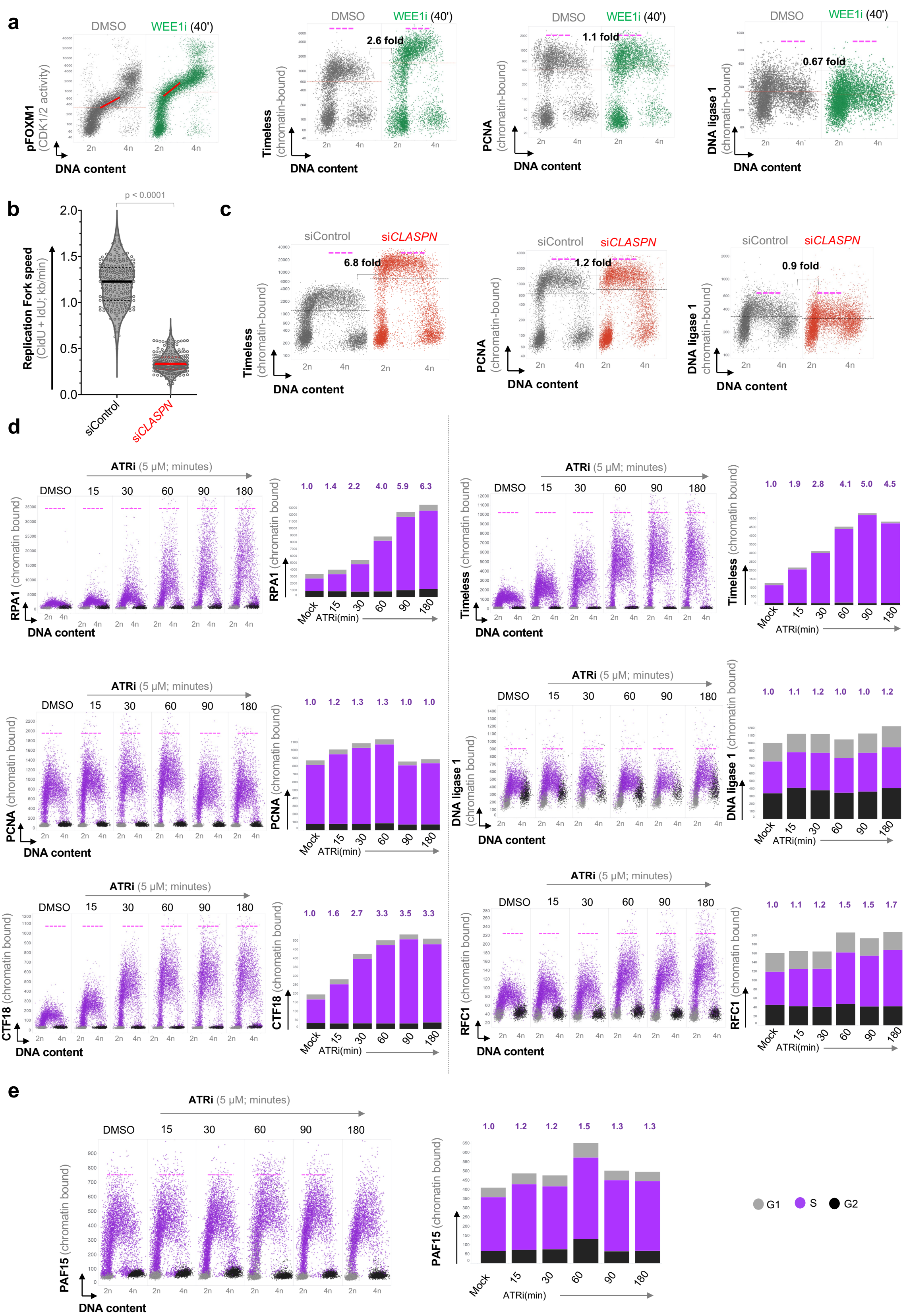
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authors and unedited



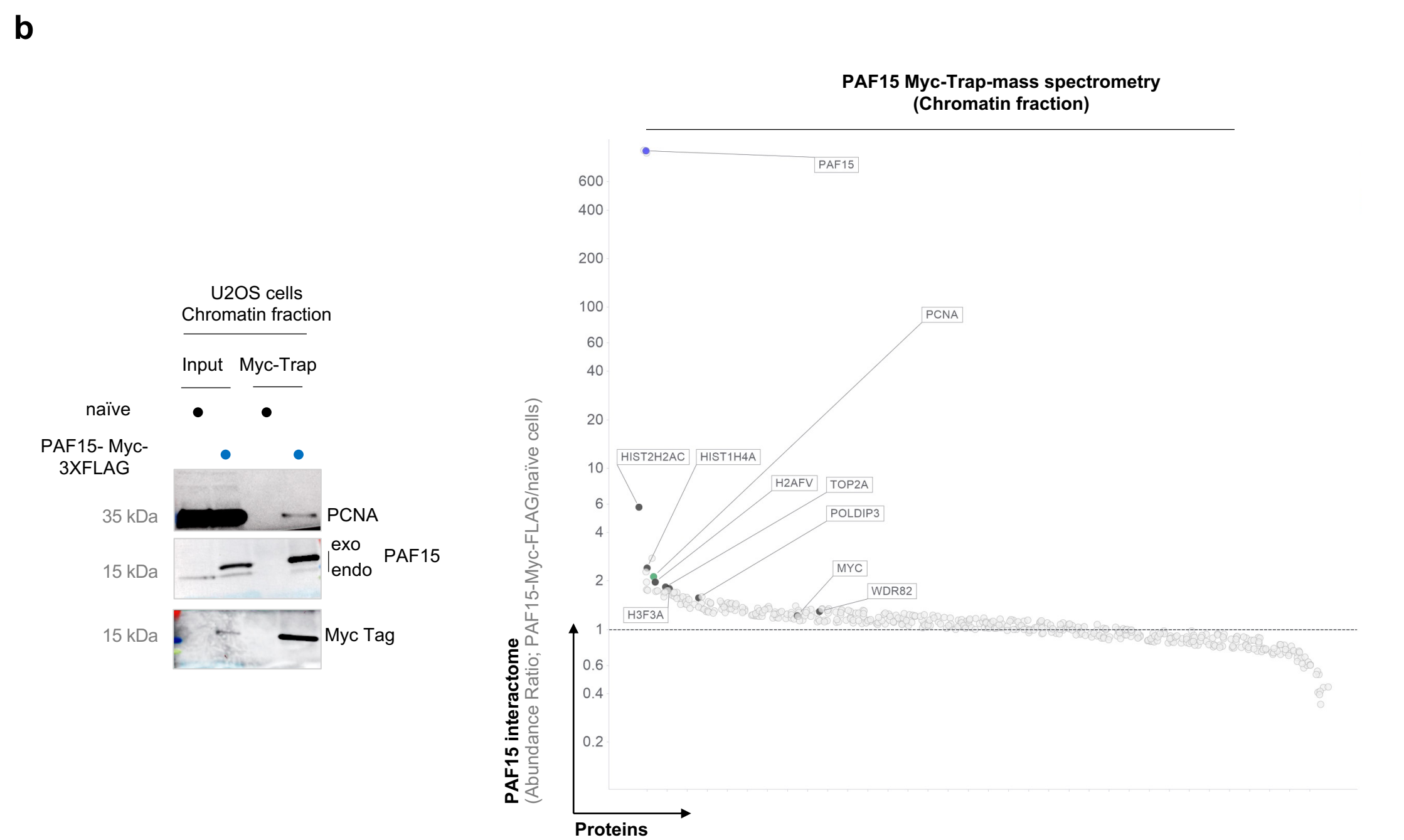
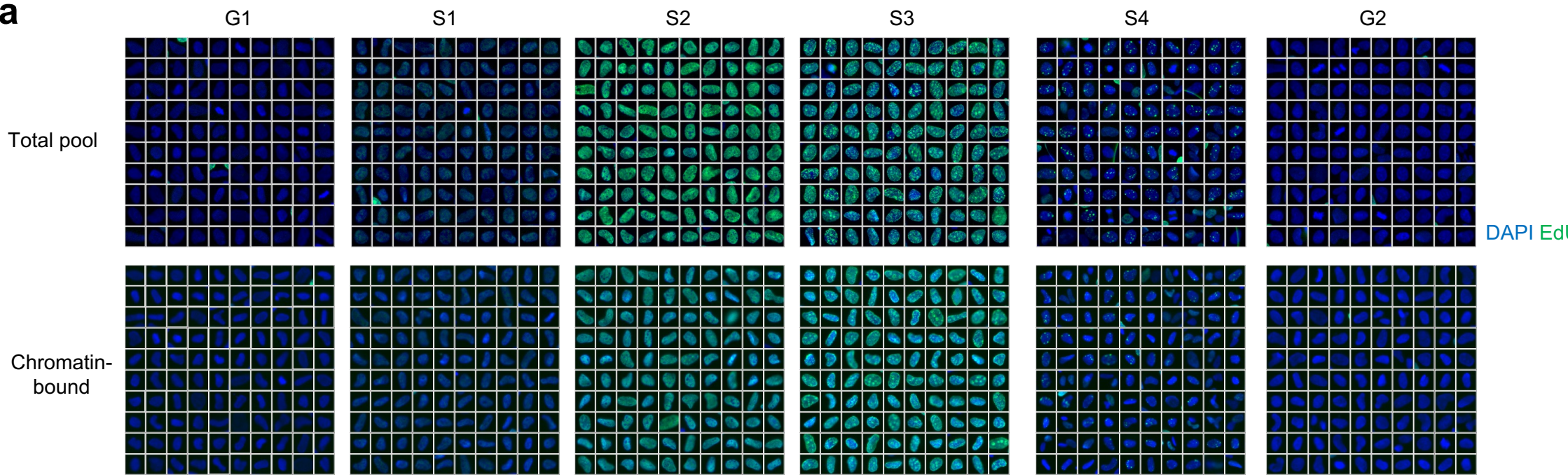
**SI Figure 1. Rapid upregulation of CDK1/2 activities and origin firing upon ATR inhibition.** **a**, Representative images from high-content microscopy of U2OS cells immunostained for phosphorylated FOXM1 (pFOXM1 T600) following ATR inhibition at the indicated timepoints. **b**, (Top) Representative high-content microscopy images of U2OS cells immunostained for  $\gamma$ H2AX following ATR inhibition at the indicated timepoints. **c**, Representative time-lapse snapshots of U2OS cells stably expressing the PCNA TagRFP-chromobody following the indicated treatments and at specified timepoints. Scale bar, 10  $\mu$ m. See also Fig. 1a for quantifications of the PCNA chromobody at early timepoints. All the experimental data are derived from minimum of 2 independent experiments. **d**, 3D confocal microscopy image of U2OS cells expressing endogenously tagged CDC45-GFP and immunostained for GFP and PCNA following exposure to an ATR inhibitor as described in **Fig. 1b**. Scale bar, 10  $\mu$ m.





**SI Figure 2. Natural depletion of chromatin-associated PCNA-lagging strand factors is consistent across cell types and mode of CDK1/2 upregulation.** **a**, (Left) QIBC analysis of pFOXM1 in DMSO and WEE1 inhibitor (Adavosertib, 10  $\mu$ M) treated in U2OS cells. n >10,000 cells per condition. (Middle and Right) QIBC analysis of chromatin-bound Timeless, PCNA, and DNA ligase 1 in U2OS cells treated with WEE1 inhibitor. n >5,000 cells per condition. **b**, Replication fork speed of U2OS cells treated with control siRNA and siRNA against *Claspin*. n = 200 fibers for each condition. P values were determined by one-way ANOVA with Tukey’s test. **c**, QIBC analysis of chromatin-bound fraction of indicated proteins in U2OS cells treated with control siRNA and siRNA against *CLSPN*. 2n corresponding to G1 phase and 4n to G2 phase; >8,000 cells per condition. The horizontal lines in QIBC plot depict the average values. Pink dotted lines indicate the approximate maximum levels of each protein. **d**, QIBC analysis and cell cycle-specific quantification of indicated chromatin-bound proteins during ATR inhibition at indicated timepoints. 2n corresponding to G1 phase and 4n to G2 phase; >3,000 cells per condition. **Fig 1g** is derived from the average values of the indicated ATRi time points for each chromatin-bound protein. **e**, QIBC analysis and cell cycle-specific quantification of chromatin-bound PAF15 during ATR inhibition at indicated timepoints. 2n corresponding to G1 phase and 4n to G2 phase; >3,000 cells per condition. All QIBC measurements were quantified in arbitrary units (A.U.). Each QIBC plot shown is representative of at least two independent biological replicates.





**SI Figure 3. PAF15 chromatin interactome.** **a**, Gallery images of EdU staining under fixed (total) and pre-extracted (chromatin) and cell cycle stage stratifications (G1 phase, 4 S-phase quintiles (1-4) and G2 phase). **b**, (Left) Mass spectrometry analysis of the enrichment of chromatin-bound proteins trapped by Myc IP in U2OS cells.  $n = 2$  biological replicates. Note that, owing to the missing values of PAF15 peptides in naïve U2OS cells (below the detection limit of the MS), the ratio values are very high for PAF15. (Middle) Immunoblotting of Myc IP from naïve U2OS and PAF15-Myc-3X FLAG constitutive overexpression.

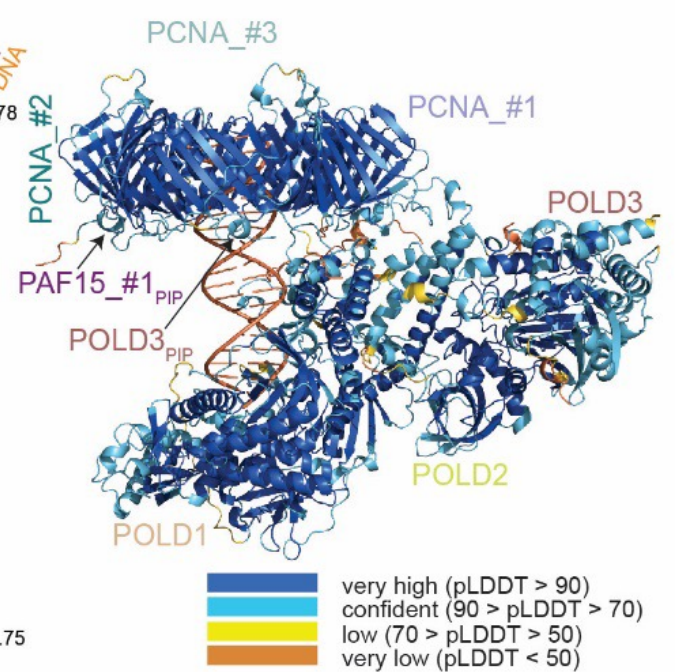
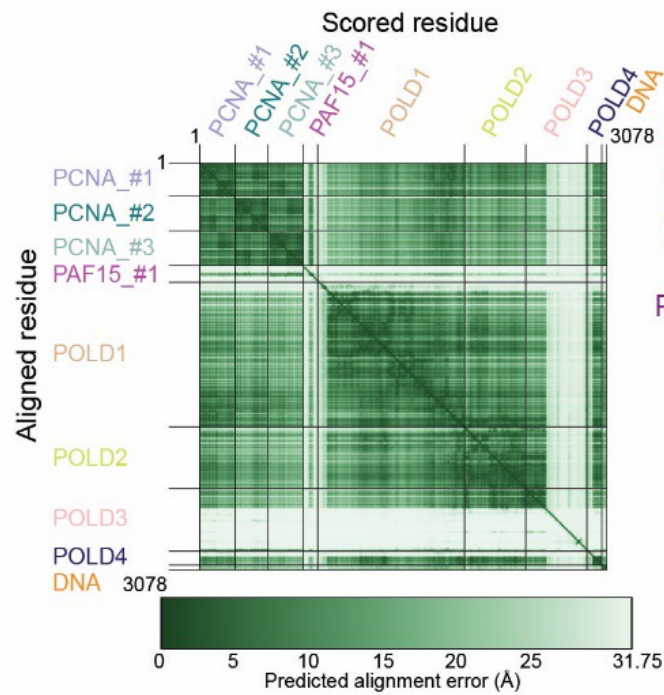
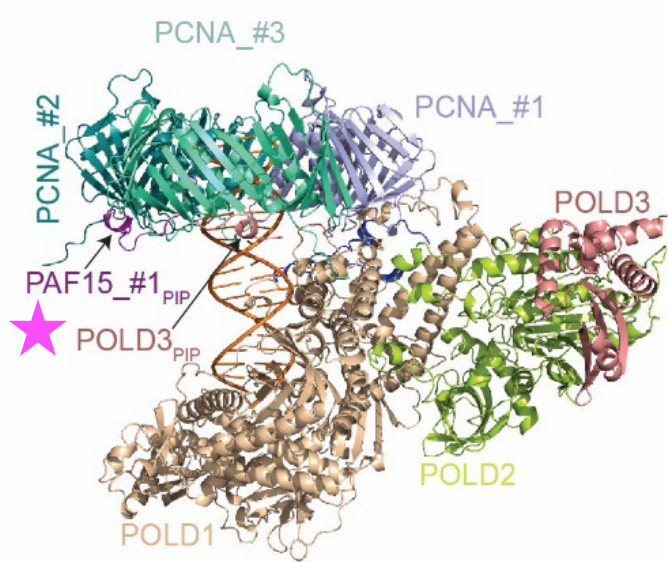


**a****PCNA trimer: DNA: POLD holocomplex: PAF15**

Model coloured by chains and trimmed for low pLDDT scores

PAE scores of the complex

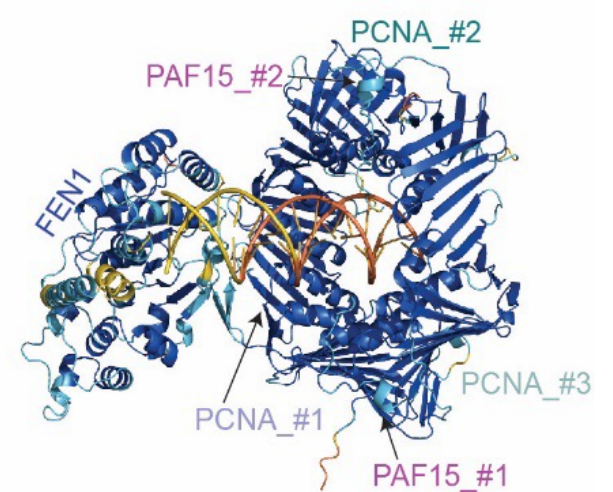
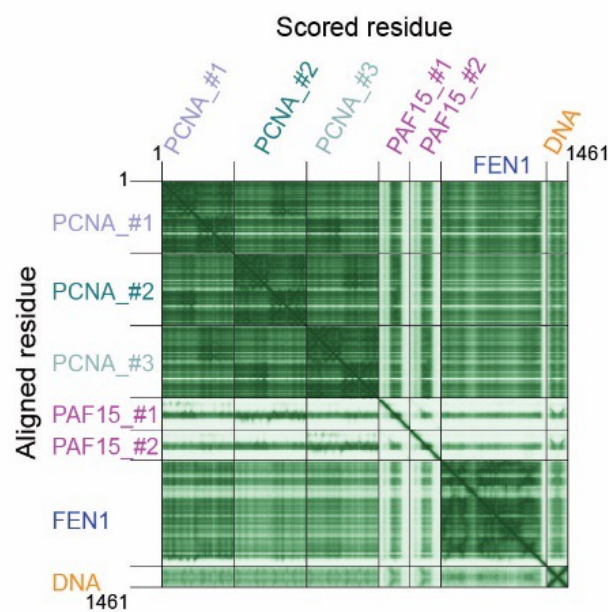
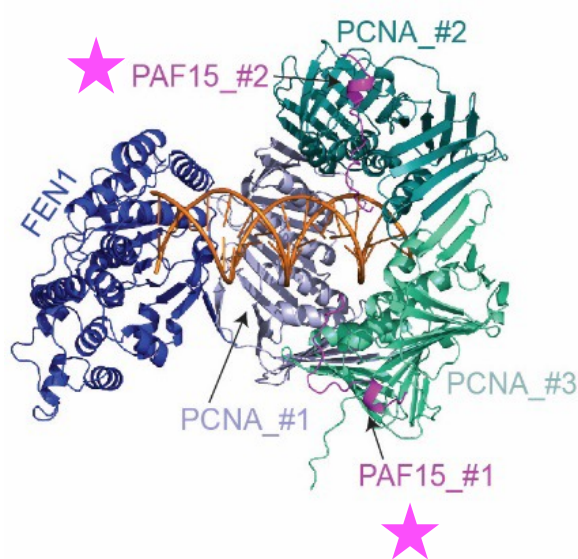
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**b****PCNA trimer: DNA: FEN1: PAF15**

Model coloured by chains and trimmed for low pLDDT scores

PAE scores of the complex

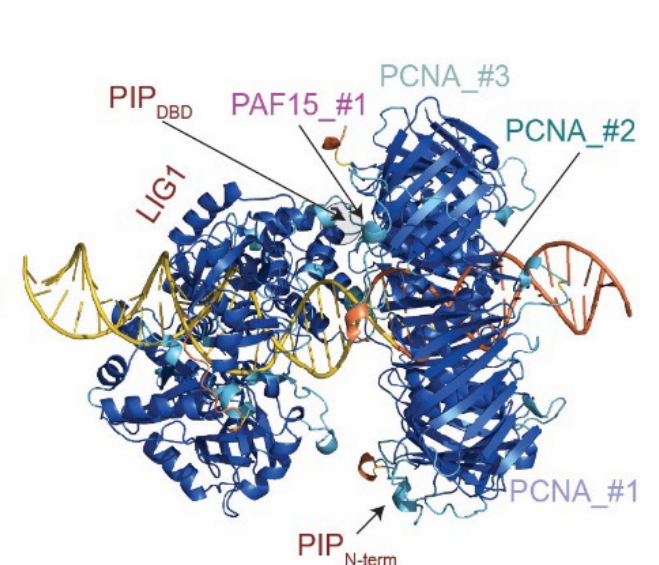
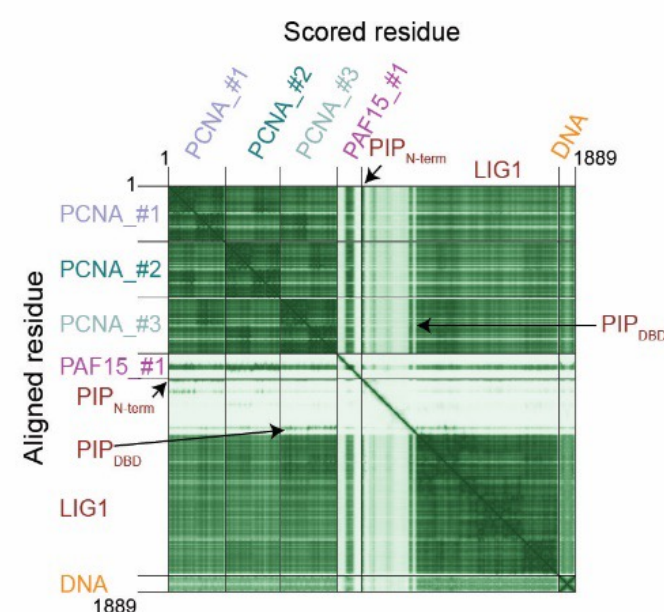
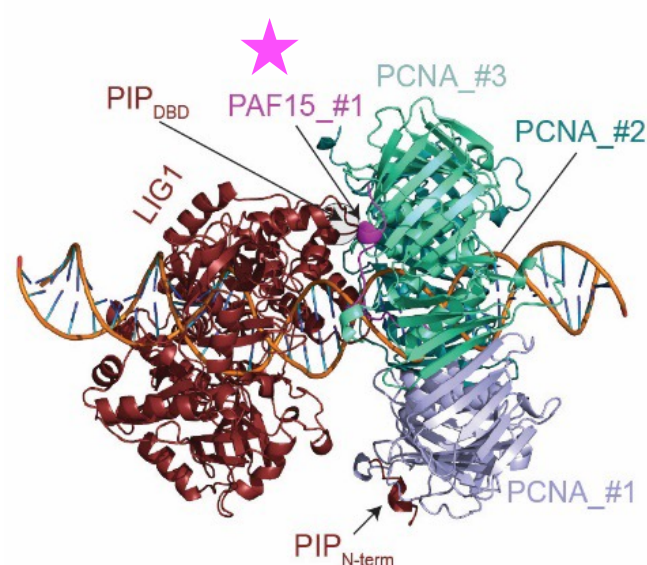
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**c****PCNA trimer: DNA: DNA ligase1: PAF15**

Model coloured by chains and trimmed for low pLDDT scores

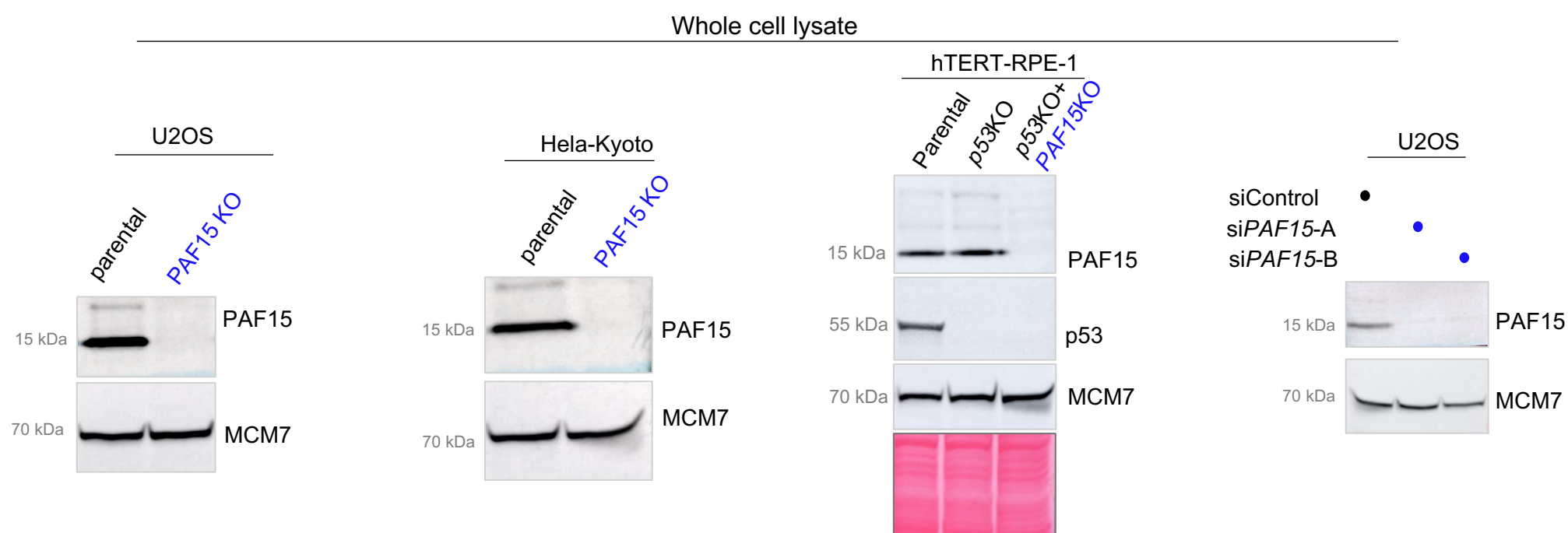
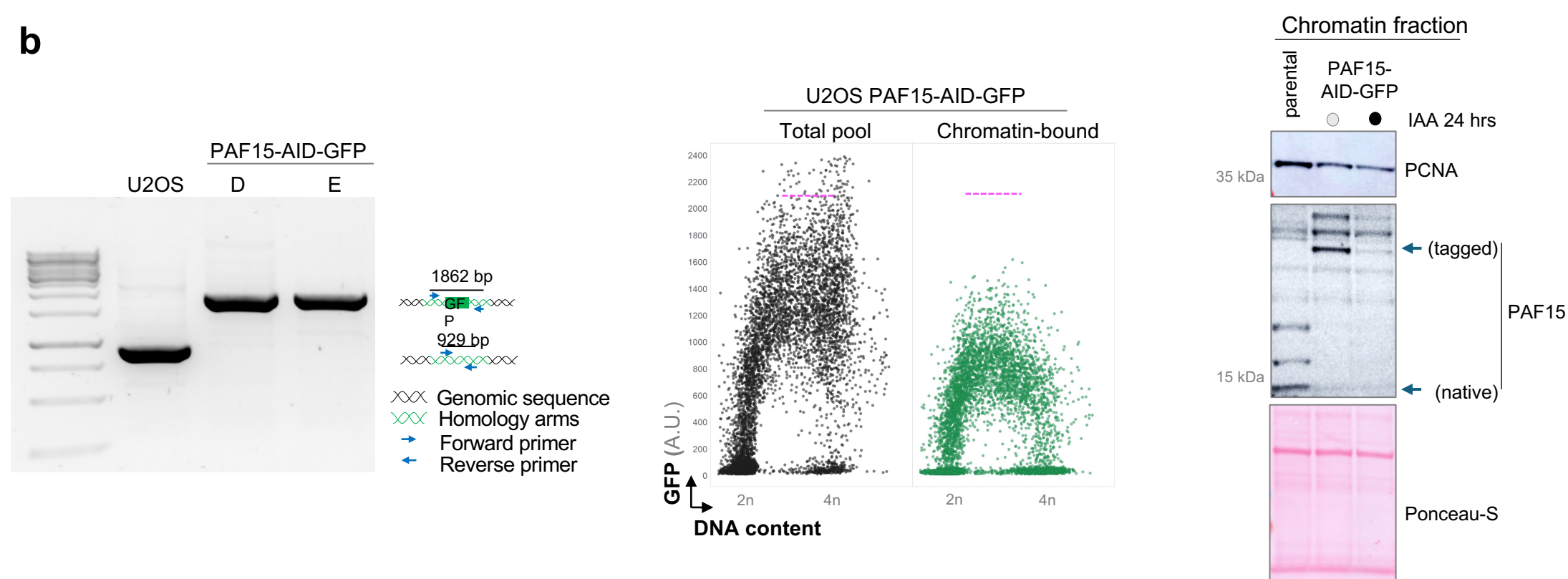
PAE scores of the complex

Model coloured by pLDDT scores



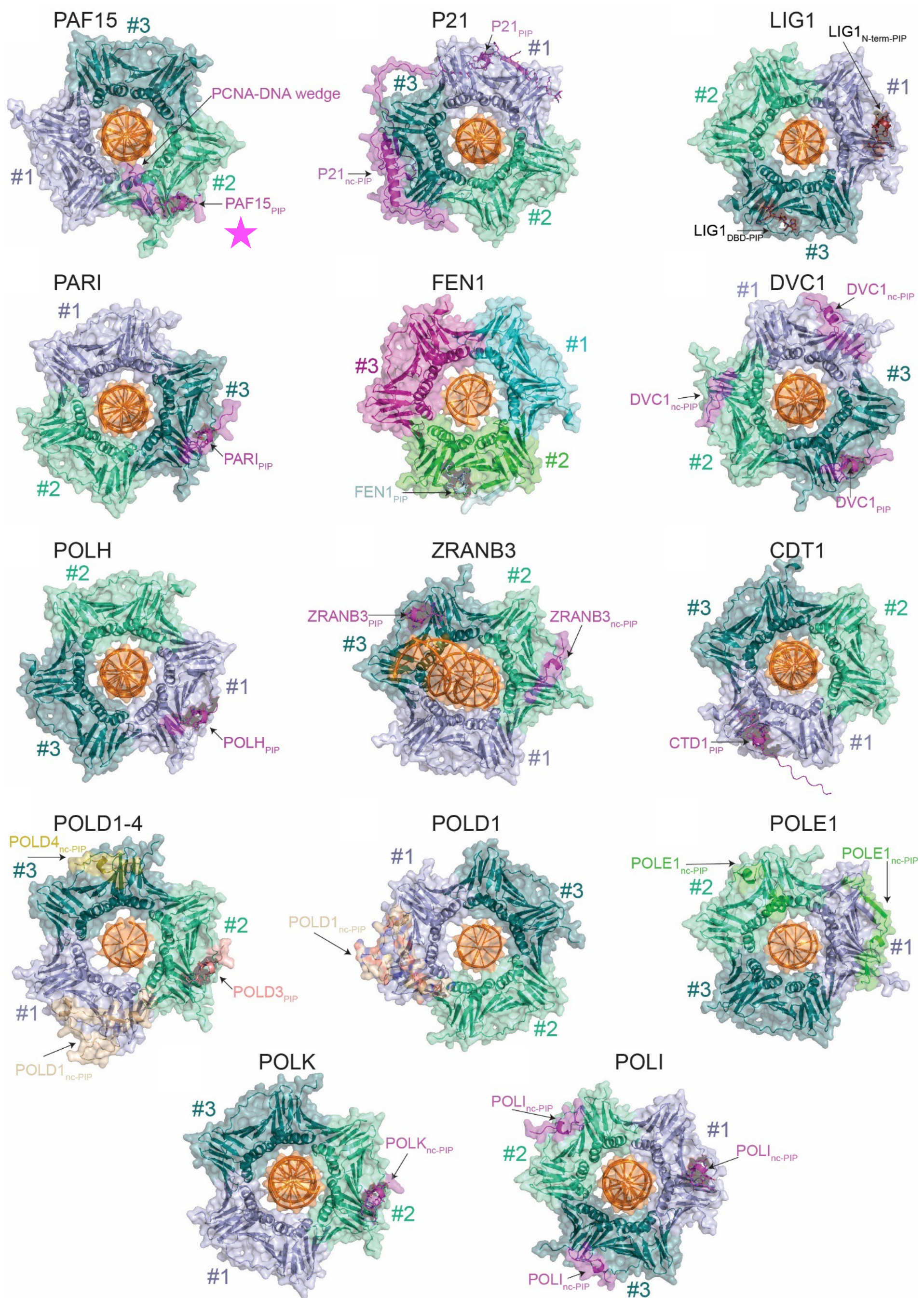
**SI Figure 4. The occupancy of PCNA by PAF15 controls the access of lagging-strand PCNA-interactors. a,** AlphaFold predicts a nonameric PCNA-PAF15-POLD-DNA complex. (Left) PAF15 occupies one PCNA monomer whilst POLD1 and POLD3 saturates the other two. The model is predicted with high confidence as revealed by predicted alignment errors (PAE<10Å, Middle) and high per-residues confidence scores (pLDDT>90, Right). Few intrinsically disordered regions that comprises the residues 1-74 of POLD1, 457-467 of POLD2, 144-455 of POLD3 and 1-40 of POLD4 are predicted with very low pLDDT scores and therefore omitted from the model for clarity. **b,** The structural model of a heptameric PCNA-PAF15-FEN1-DNA shows that docking of FEN1 to one PCNA monomer allows PAF15 two occupy the other two (Left). The model is predicted with very high confidence as highlighted by low PAEs (Middle) and high pLDDTs (Right). **c,** AlphaFold predicts a hexameric PCNA-PAF15-DNA ligase 1-DNA complex where DNA ligase 1 binds the PCNA trimer by its PIPN-terminus and PIPDBD domains allowing one PAF15 molecule to access the unoccupied PCNA (Left). The model is predicted with high confidence as revealed by low PAE scores (Middle) and high pLDDT scores (Right). Intrinsically disordered regions present at the N- and C- termini of PAF15 and between the residues 1-260 of DNA ligase 1 are predicted with very low pLDDT scores and thus omitted from the model for clarity.



**a****b**

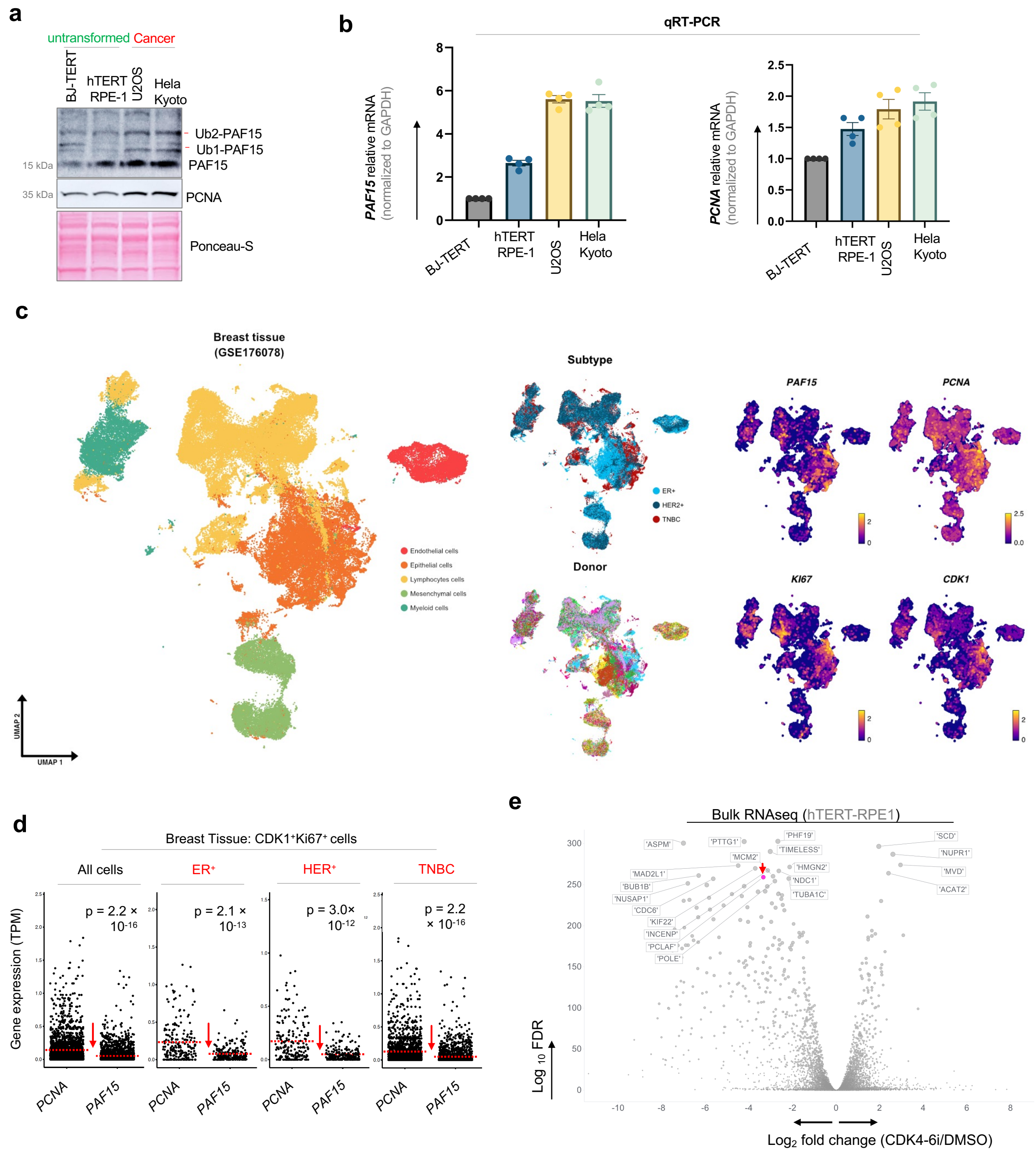
**SI Figure 5. Generation of PAF15 KO and PAF15-GFP-AID cells.** **a**, Immunoblot validation of *PAF15* KO cell lines in (left) U2OS (middle left) HeLa-Kyoto (middle right) and hTERT-RPE1 *p53* KO. (Right) Immunoblot validation of two PAF15 siRNAs. Note that hTERT-RPE1 cells do not tolerate the loss of PAF15 in the long run, therefore, *p53* was knocked out to generate *PAF15* KO cells in hTERT-RPE1 cells. **b**, (Left) Junction PCR showing homozygous PAF15–AID-GFP tagging in two clones (D and E), (Middle) QIBC analysis of the PAF15-GFP-AID loading during the cell cycle. 2n corresponding to G1 phase and 4n to G2 phase; >5,000 cells per condition. Pink dotted lines indicate the approximate maximum levels of PAF15 protein. (Right) Western blot analysis of the stability of chromatin-bound PCNA in U2OS PAF15-GFP-AID cells and upon inducible degradation of PAF15 with natural degenon indole-3-acetic acid (IAA) for 24 h.





**SI Figure 6. Unique binding mode of PAF15 with PCNA-DNA complexes.** AlphaFold simulation of the binding of multiple canonical and non-canonical PCNA-interaction partners. Apart from PAF15 (marked with a star), no other molecules are capable of transversing the inner ring of PCNA together with DNA.

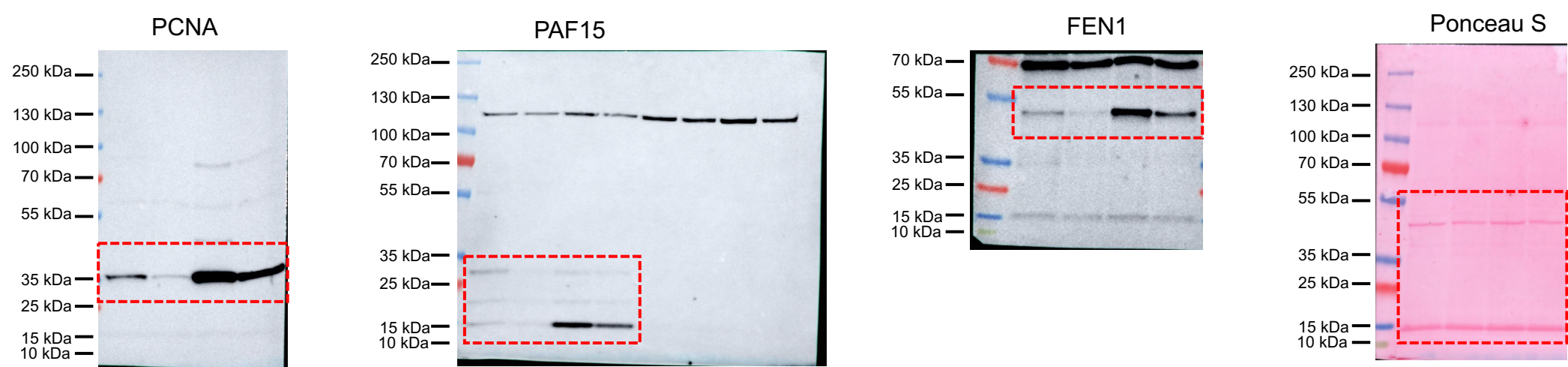




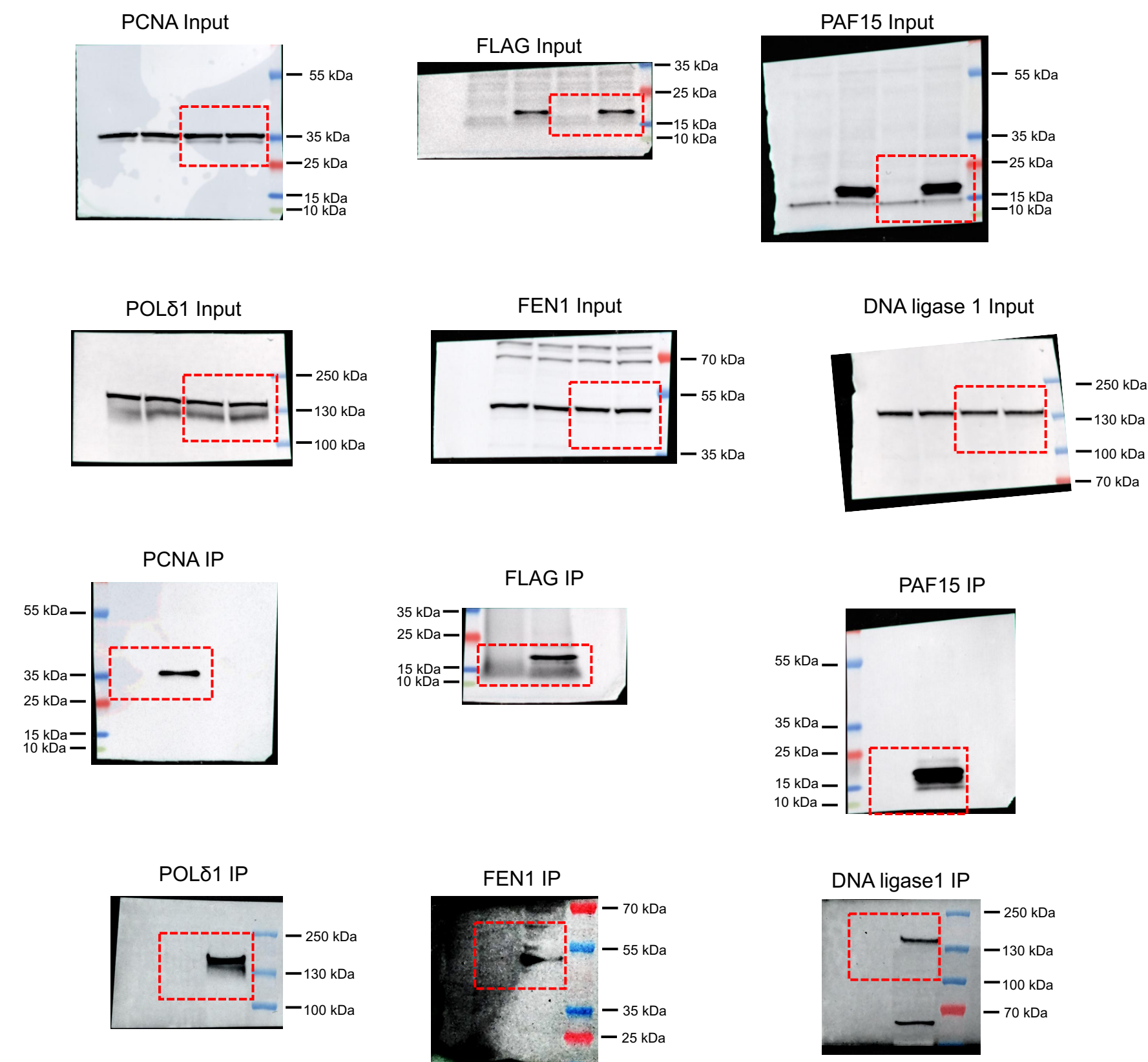
**SI Figure 7. PAF15-PCNA expression in normal and cancer cells.** **a**, Immunoblot of whole cell extract from the untransformed and cancerous cell lines comparing the PAF15 level. **b**, Relative mRNA level of PAF15 and PCNA in the indicated four different cell lines. (mean  $\pm$  s.d.; n=4) **c**, (Left) UMAP and cell type annotation of cells (n = 100,064) from public repository GSE176078 containing cells from 26 primary tumors from three major clinical subtypes of breast cancer (ER+ = 11, HER2+ = 5, and TNBC = 10). (Right) UMAP with cells split by cancer subtype and donor and Log1p gene expression of *PAF15*, *PCNA*, *KI67*, and *CDK1*. **d**, Expression of PCNA and PAF15 in CDK1+MKI67+ cells in ER+, HER2+, and TNBC breast cancer subtypes. Red dotted lines denote mean expression values. Expression values are shown as transcripts per million (TPM) with each dot representing a cell. **e**, Bulk RNAseq analysis of hTERT-RPE1 treated with 10  $\mu$ M CDK4/6 inhibitor (24 h) showing significant downregulation of PAF15 (PCLAF, red arrow).



SI Fig 8a: Uncropped immunoblot from Figure 1h



SI Fig 8b: Uncropped immunoblot from Figure 2h

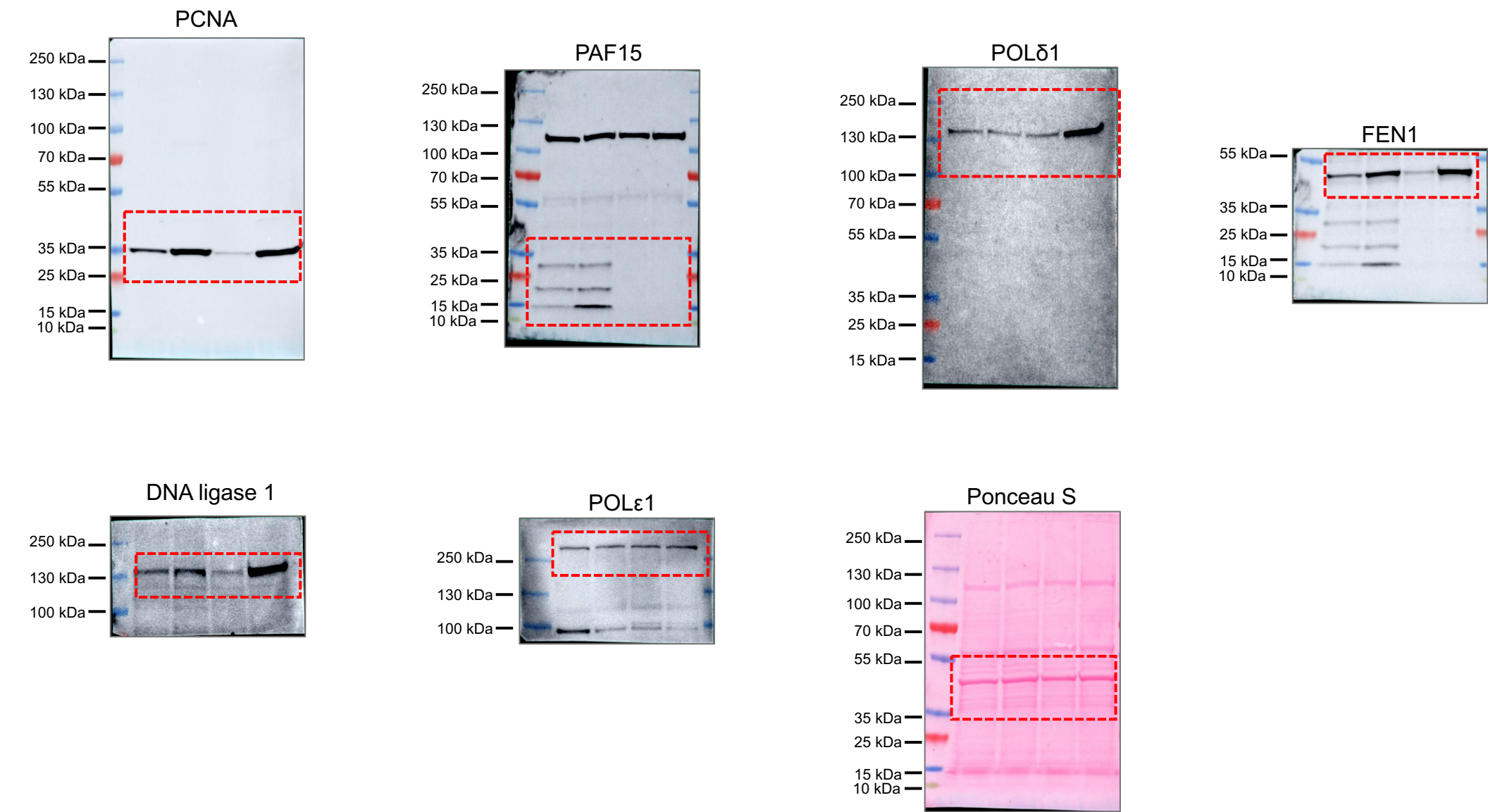




SI Fig 8c: Uncropped immunoblot from Figure 3a

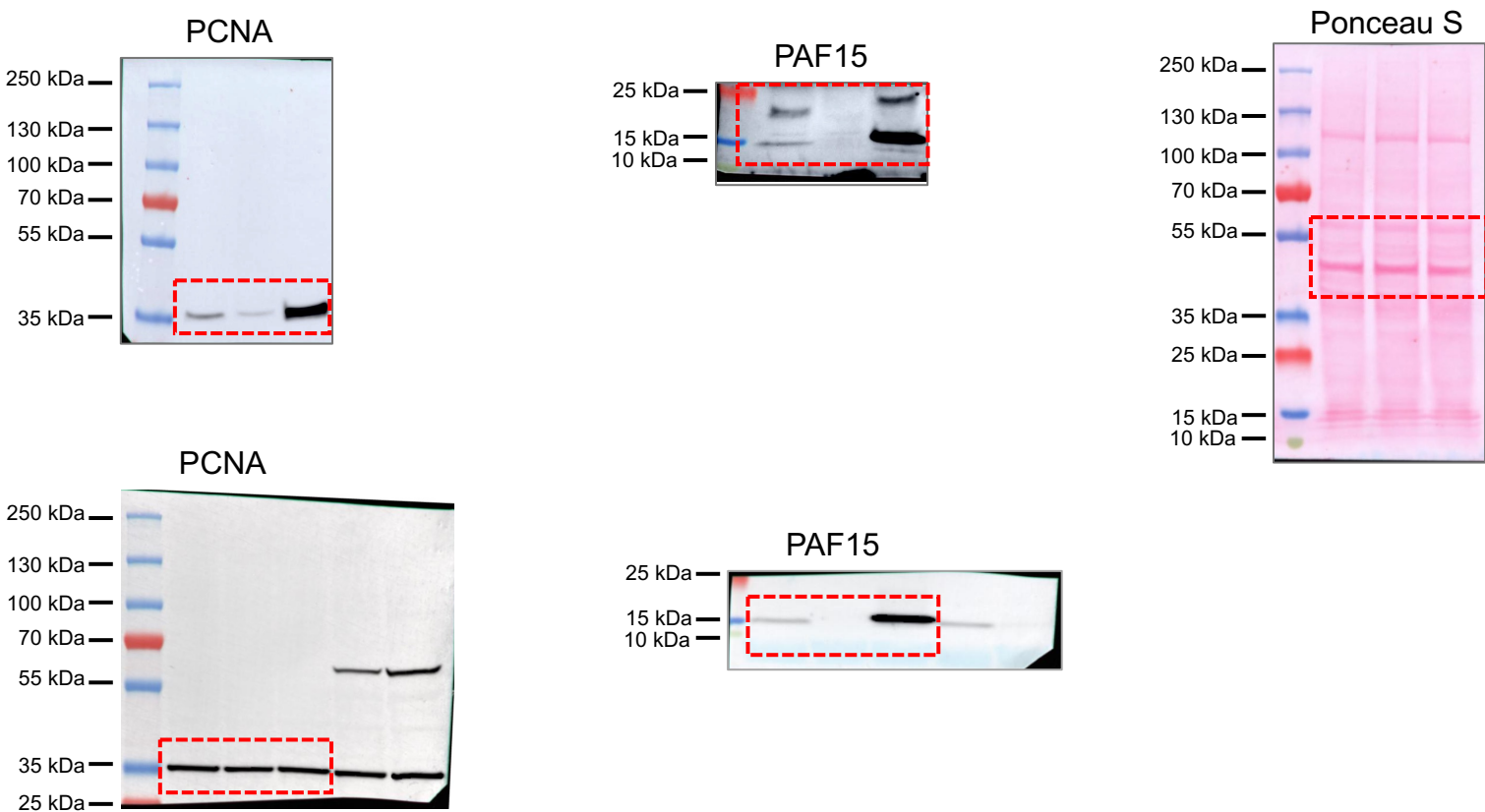


SI Fig 8d: Uncropped immunoblot from Figure 4c

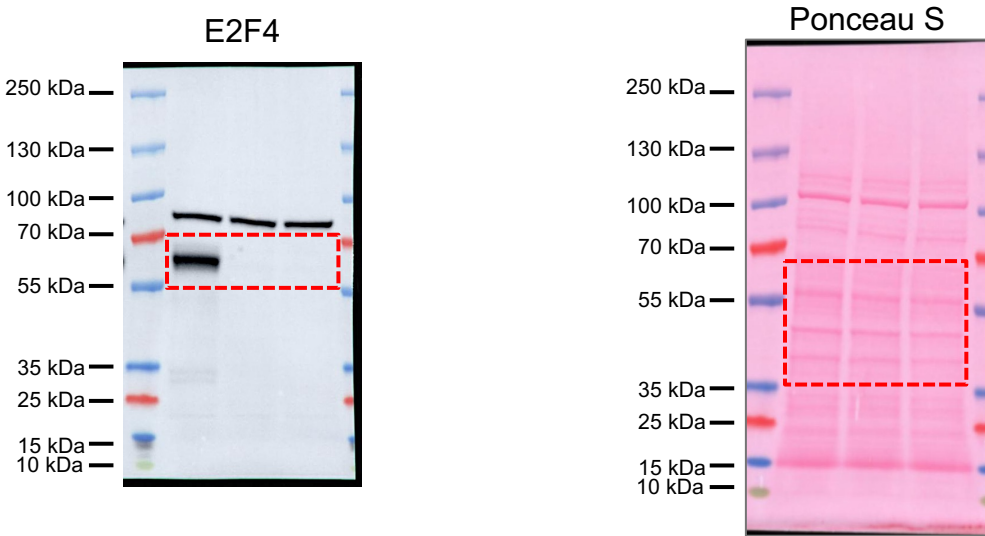




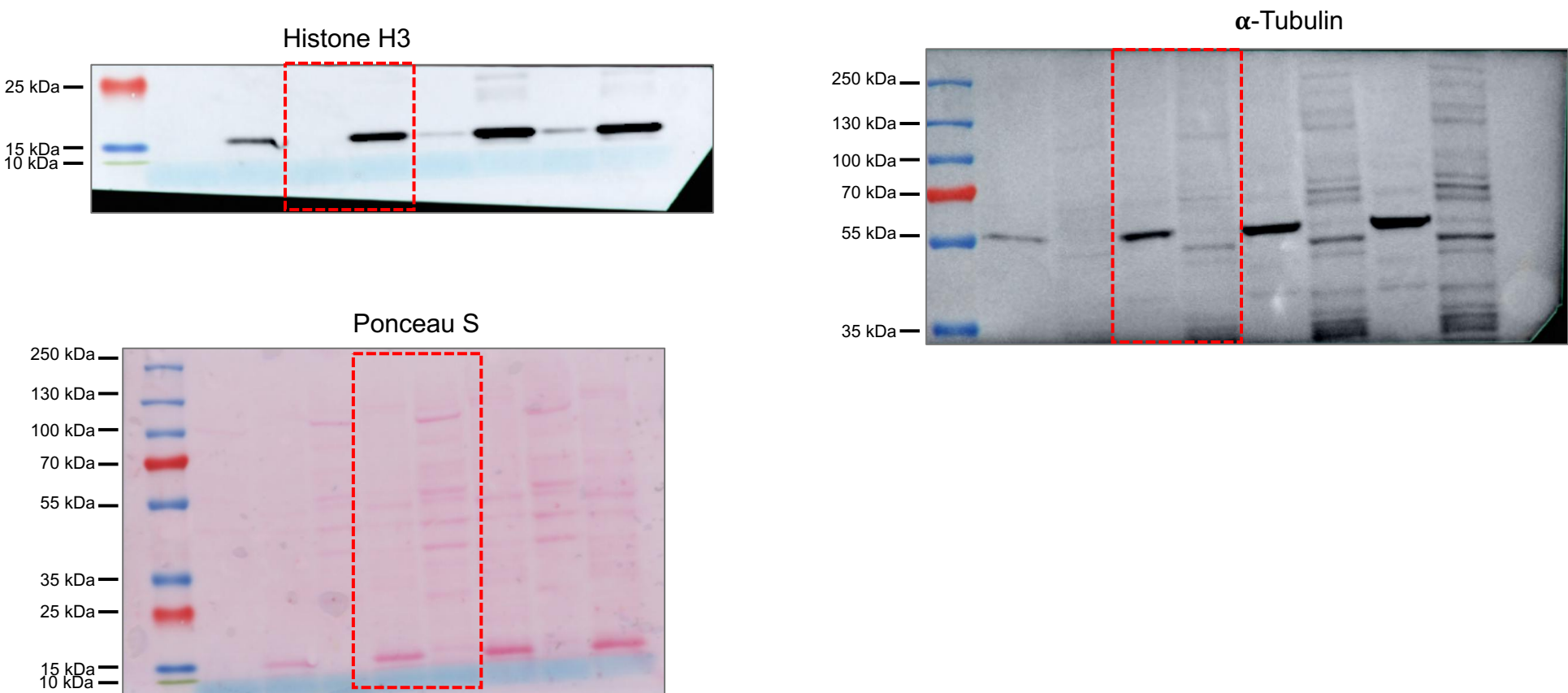
SI Fig 8e: Uncropped immunoblot from Figure 4h



SI Fig 8f: Uncropped immunoblot from Figure 5j

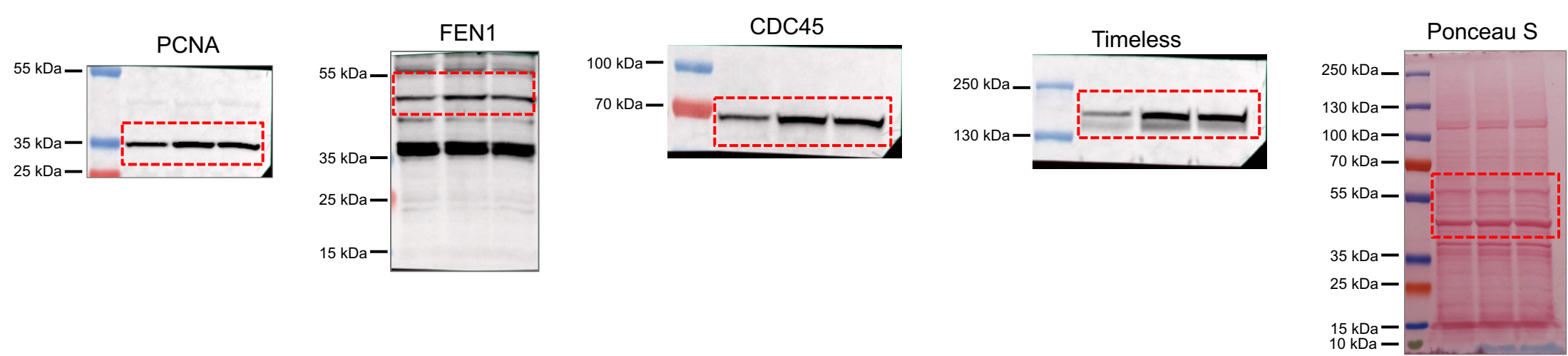


SI Fig 8g: Uncropped immunoblot from Extended Data Fig. 1h

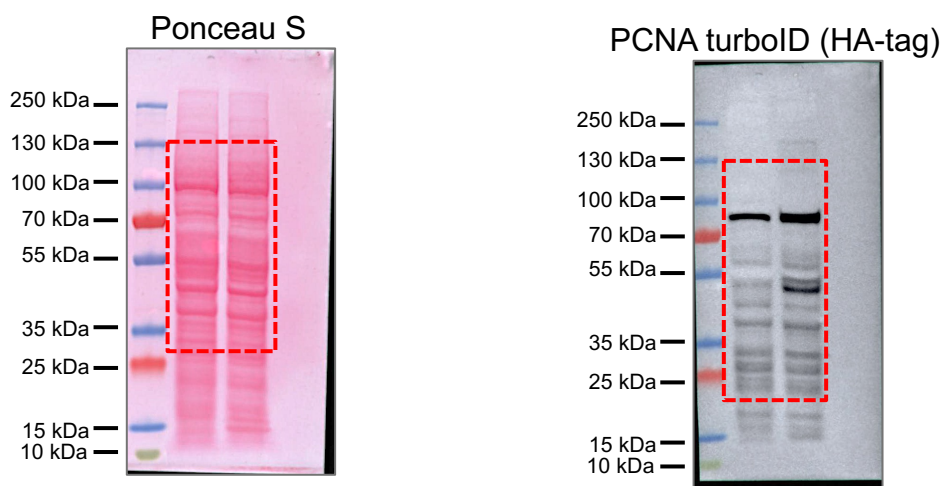




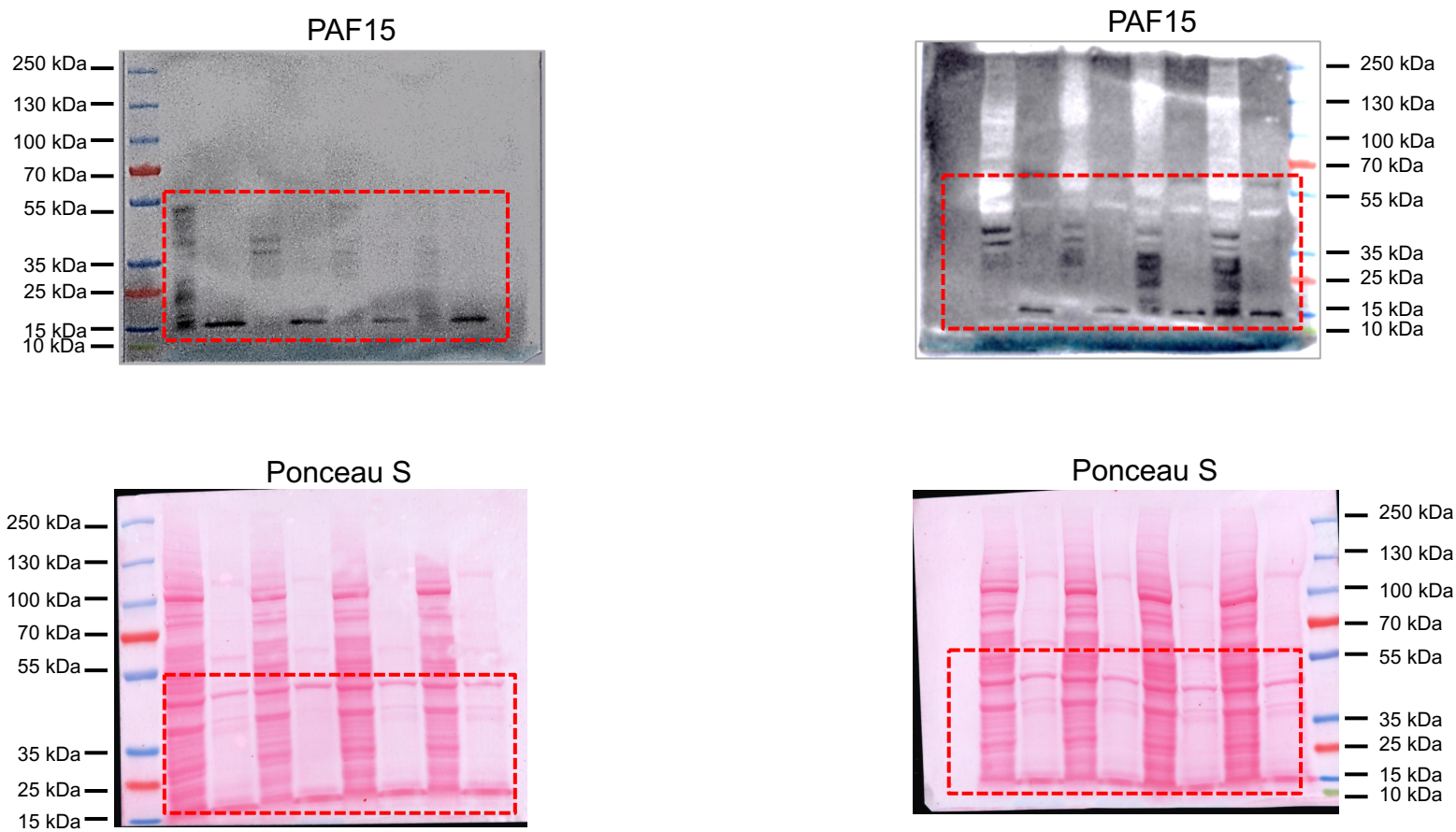
SI Fig 8h: Uncropped immunoblot from Extended Data Fig. 1i



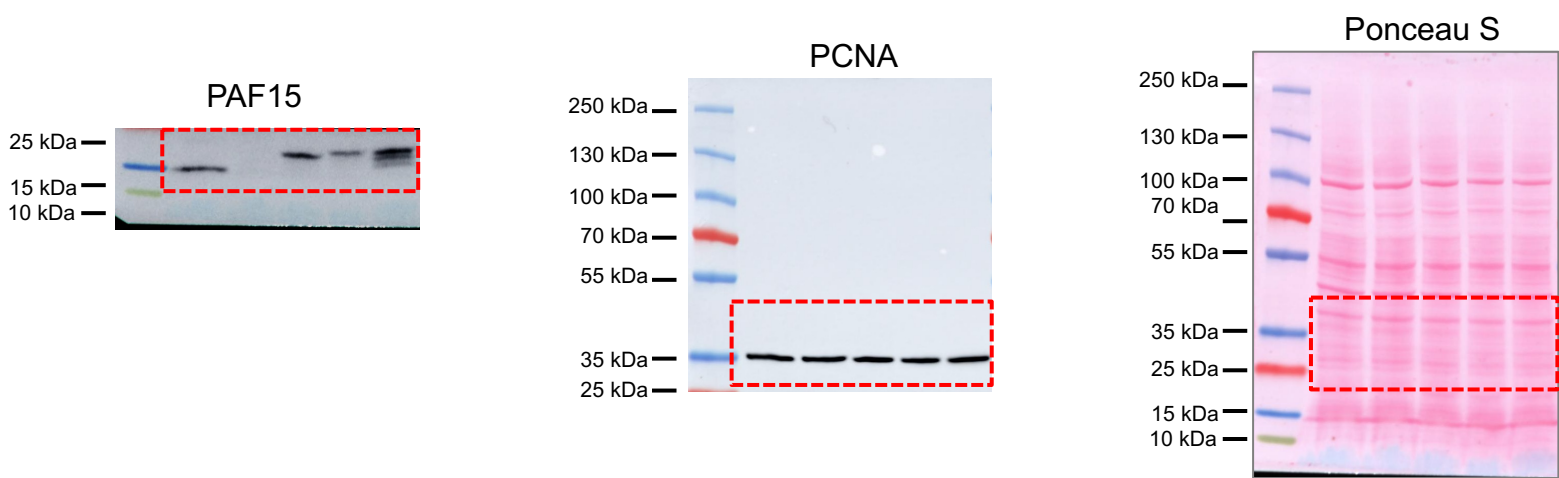
SI Fig 8i: Uncropped immunoblot from Extended Data Fig. 2f



SI Fig 8j: Uncropped immunoblot from Extended Data Fig. 3i

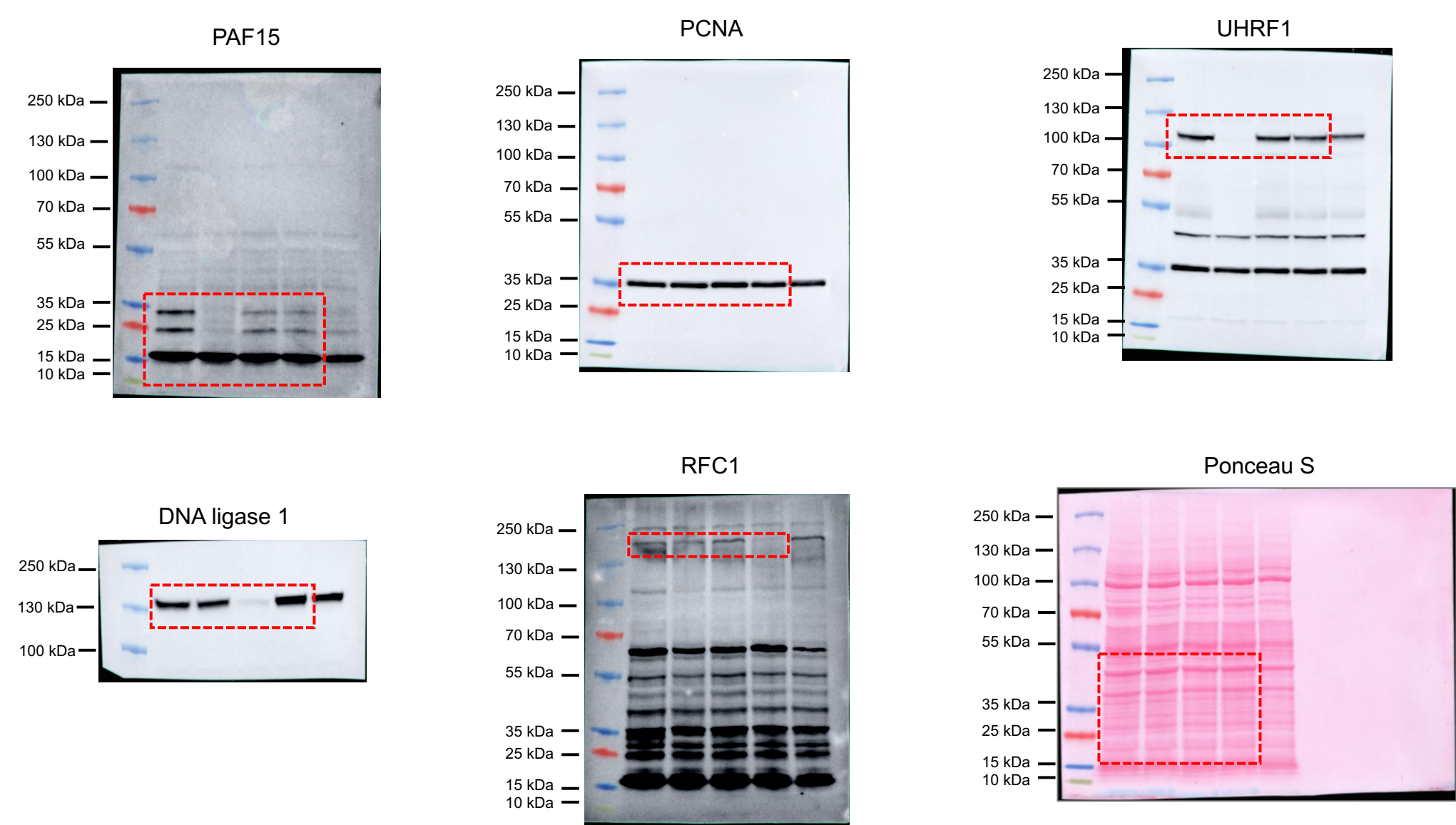


SI Fig 8k: Uncropped immunoblot from Extended Data Fig. 4a

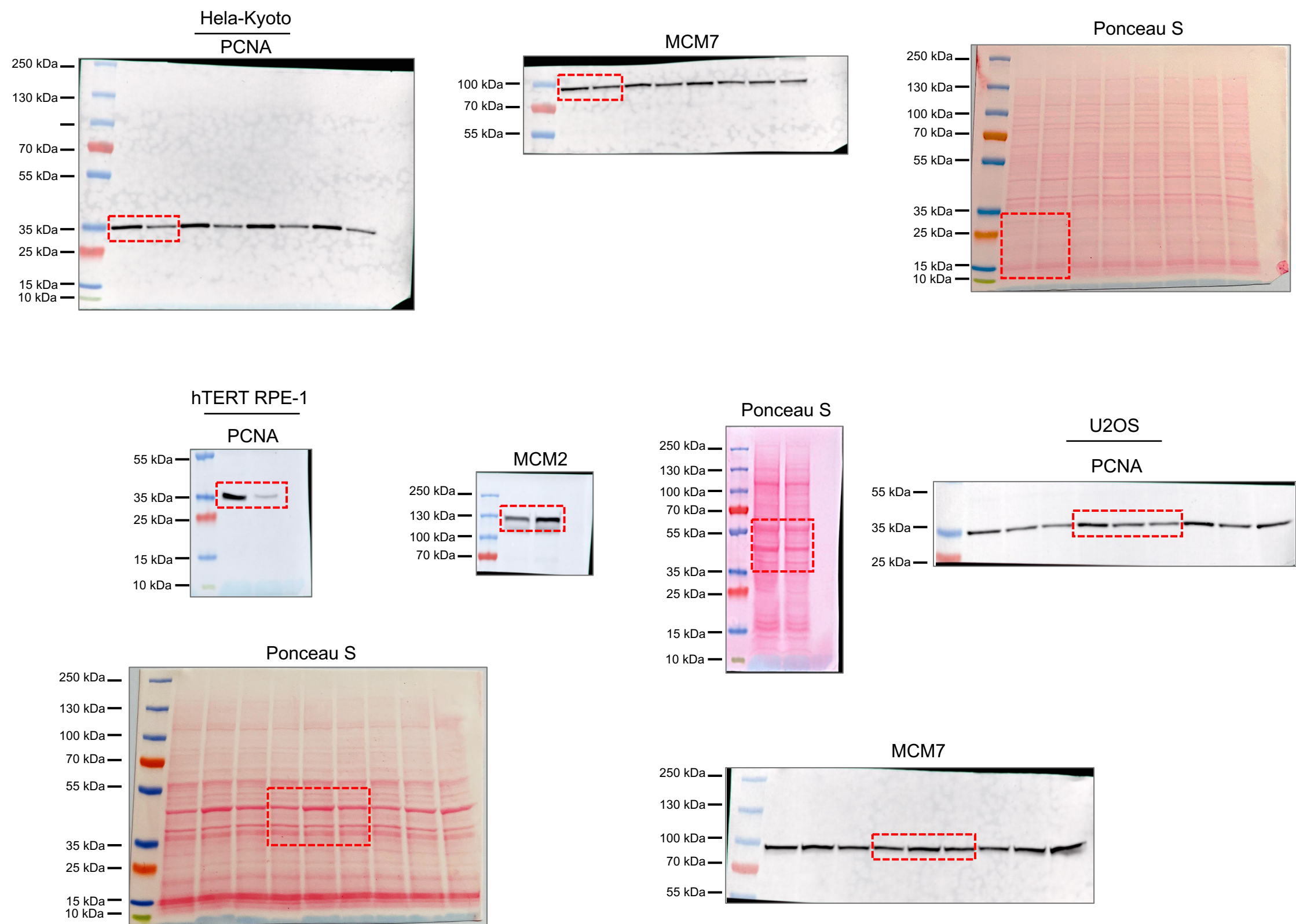




SI Fig 8l: Uncropped immunoblot from Extended Data Fig. 5a

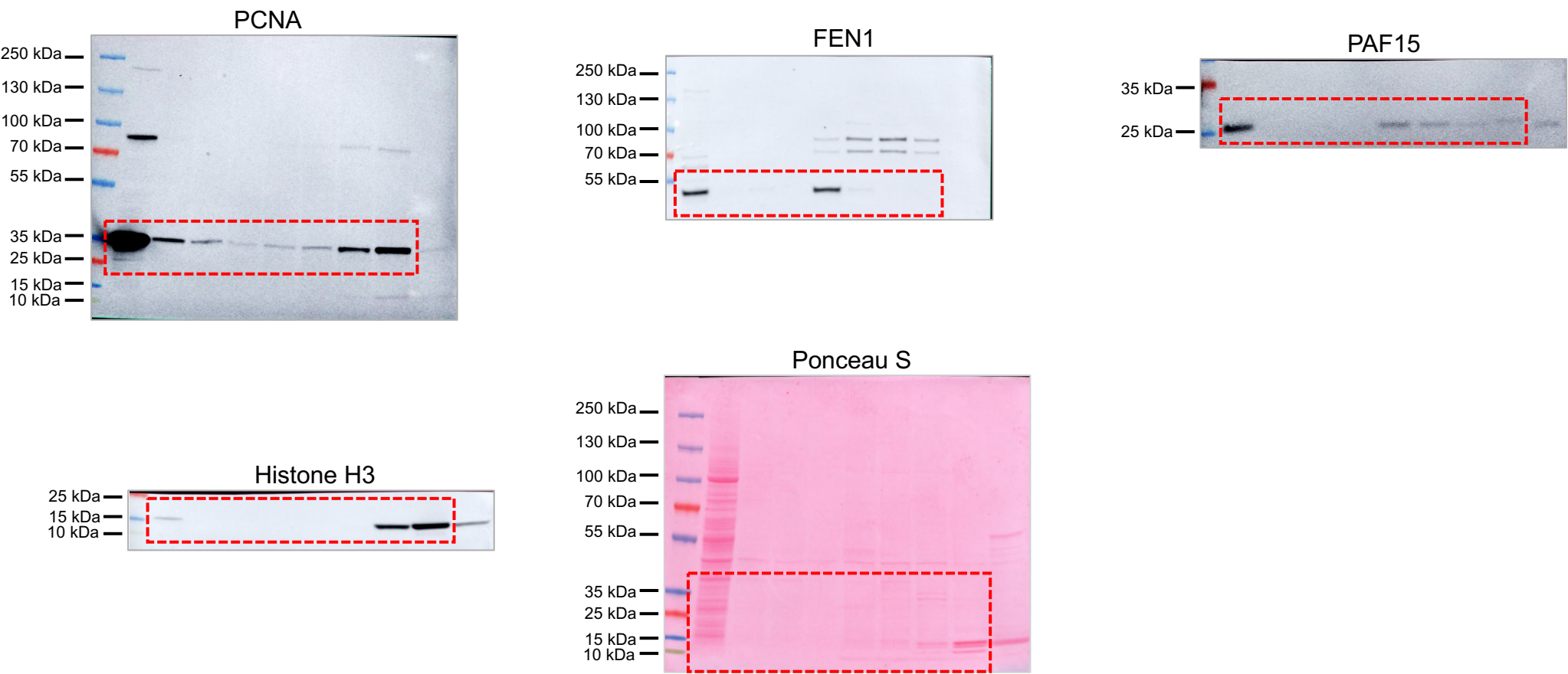


SI Fig 8m: Uncropped immunoblot from Extended Data Fig. 5f

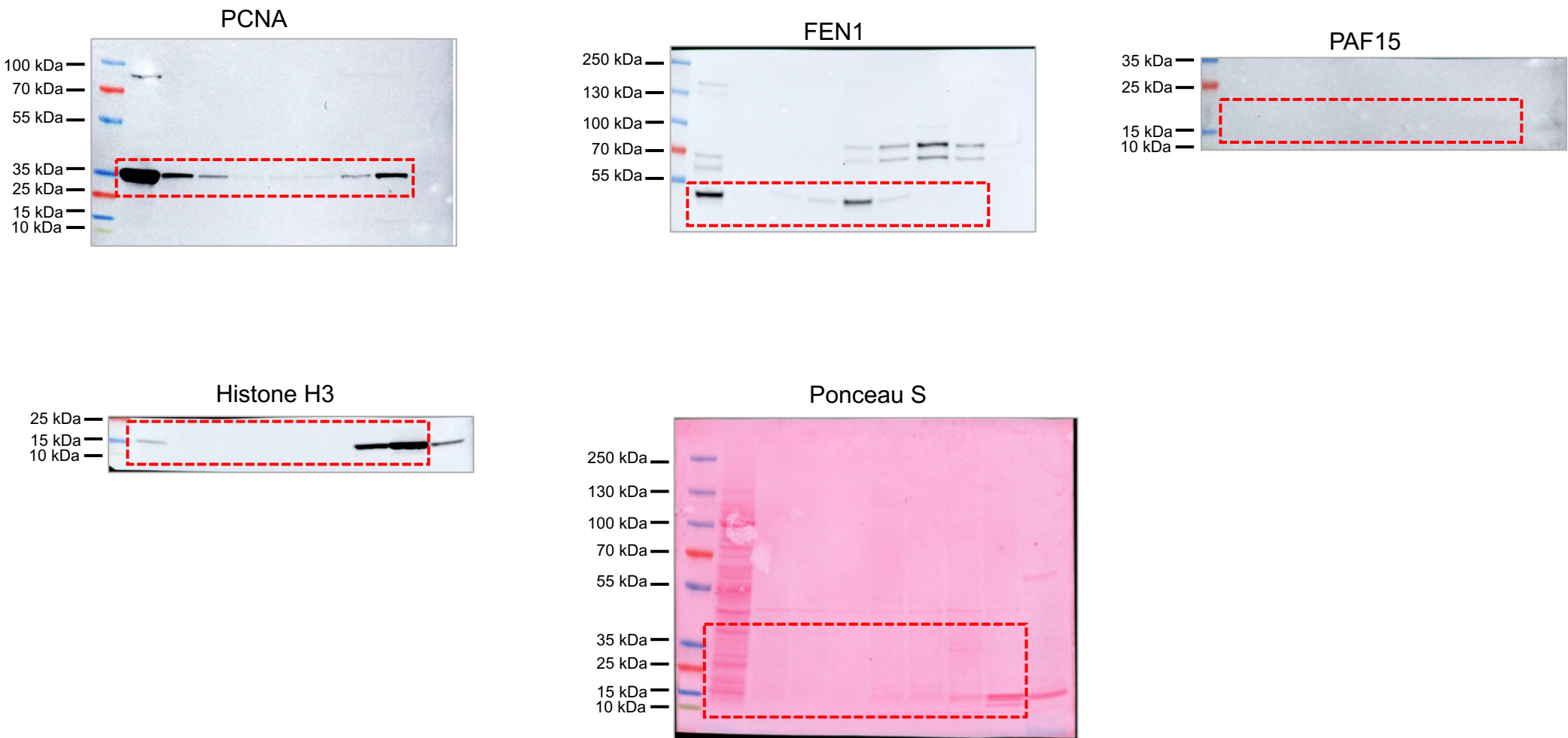


SI Fig 8n: Uncropped immunoblot from Extended Data Fig. 6i

U2OS Parental

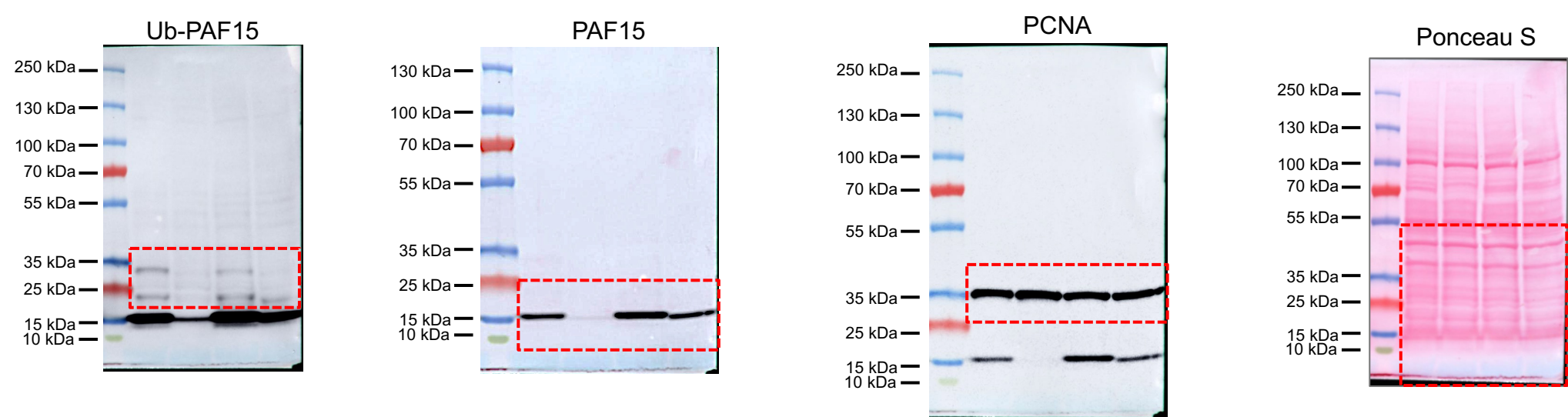


U2OS PAF15 KO

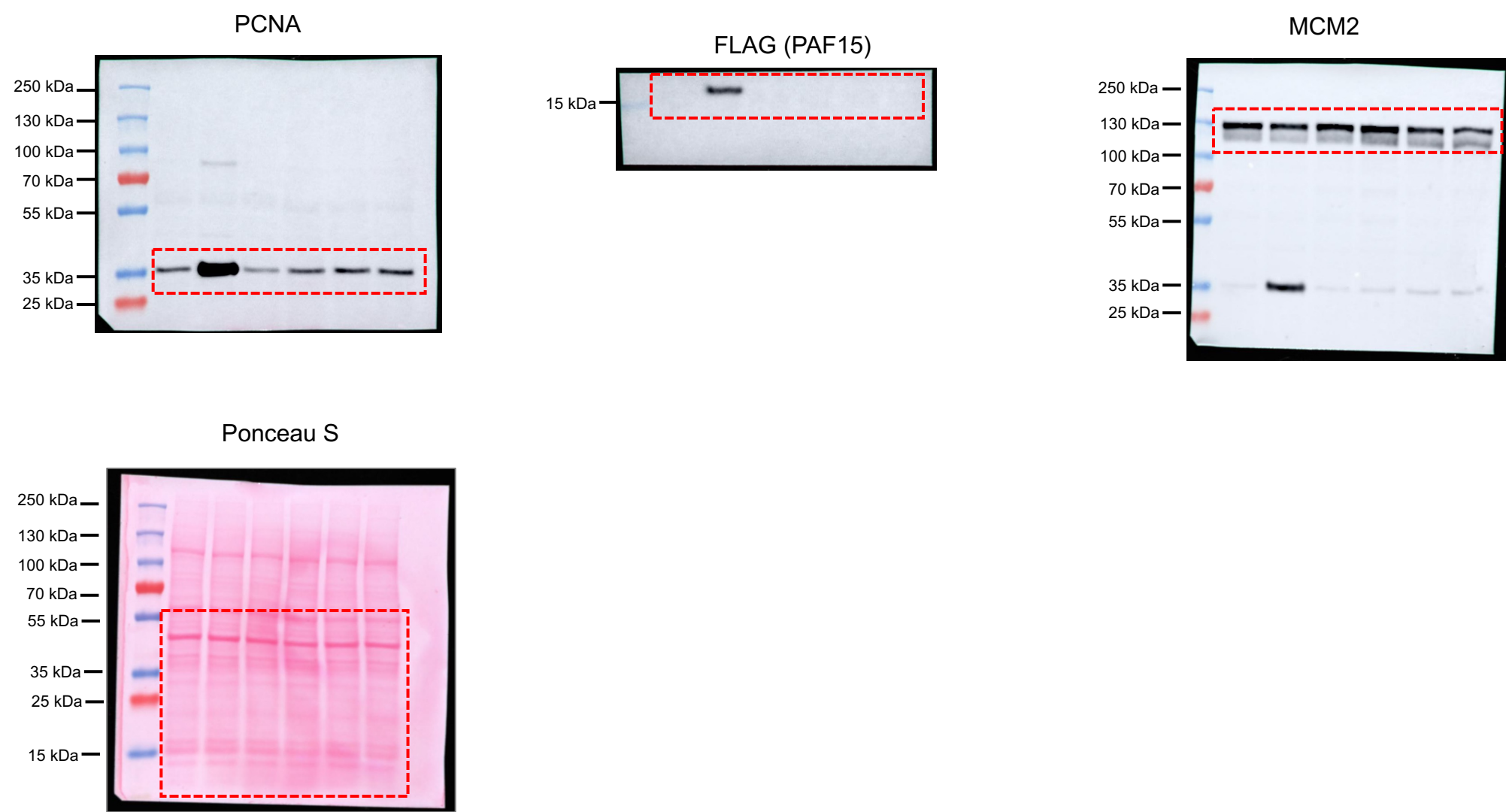




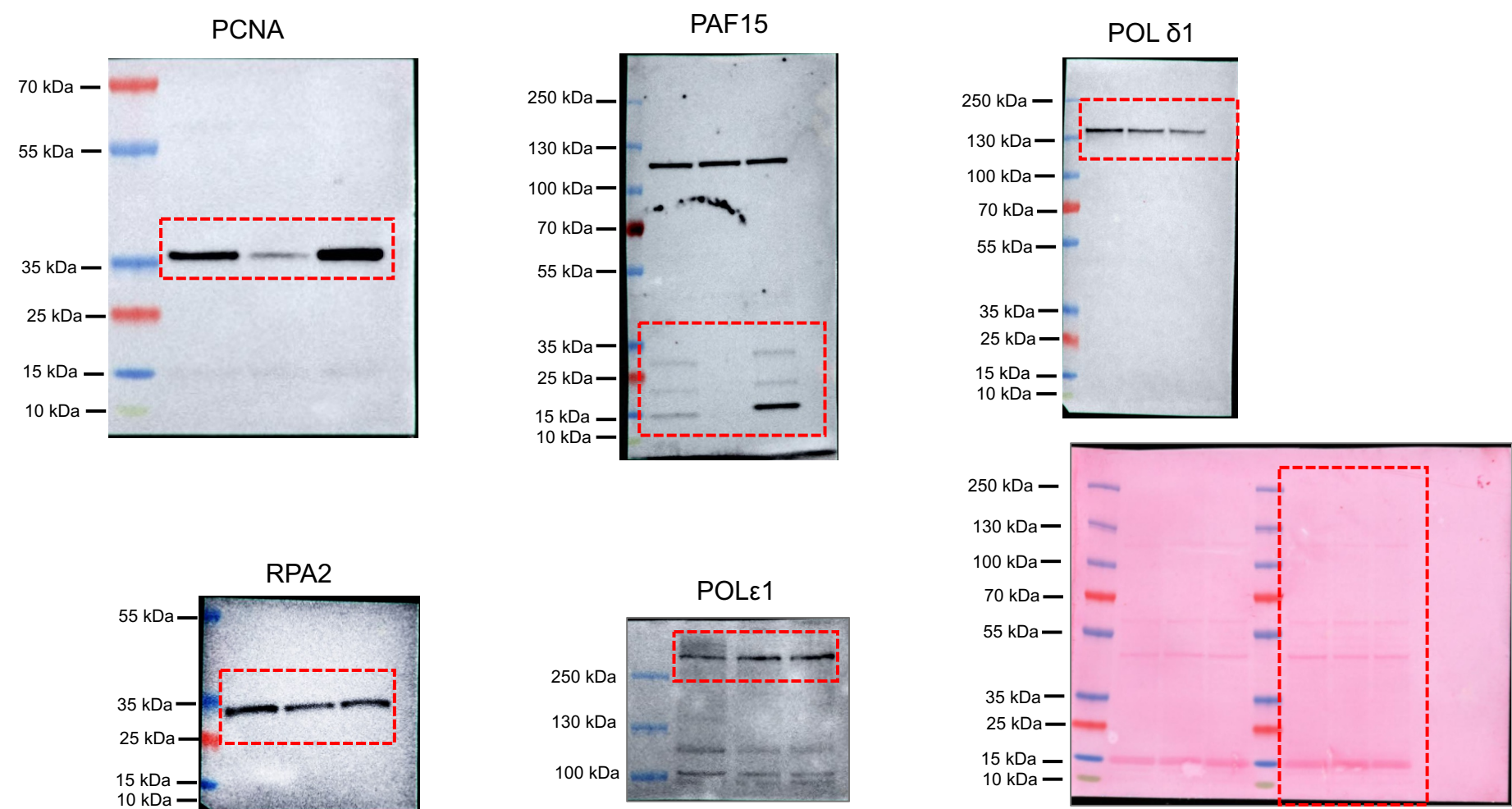
SI Fig 8o: Uncropped immunoblot from Extended Data Fig. 7g



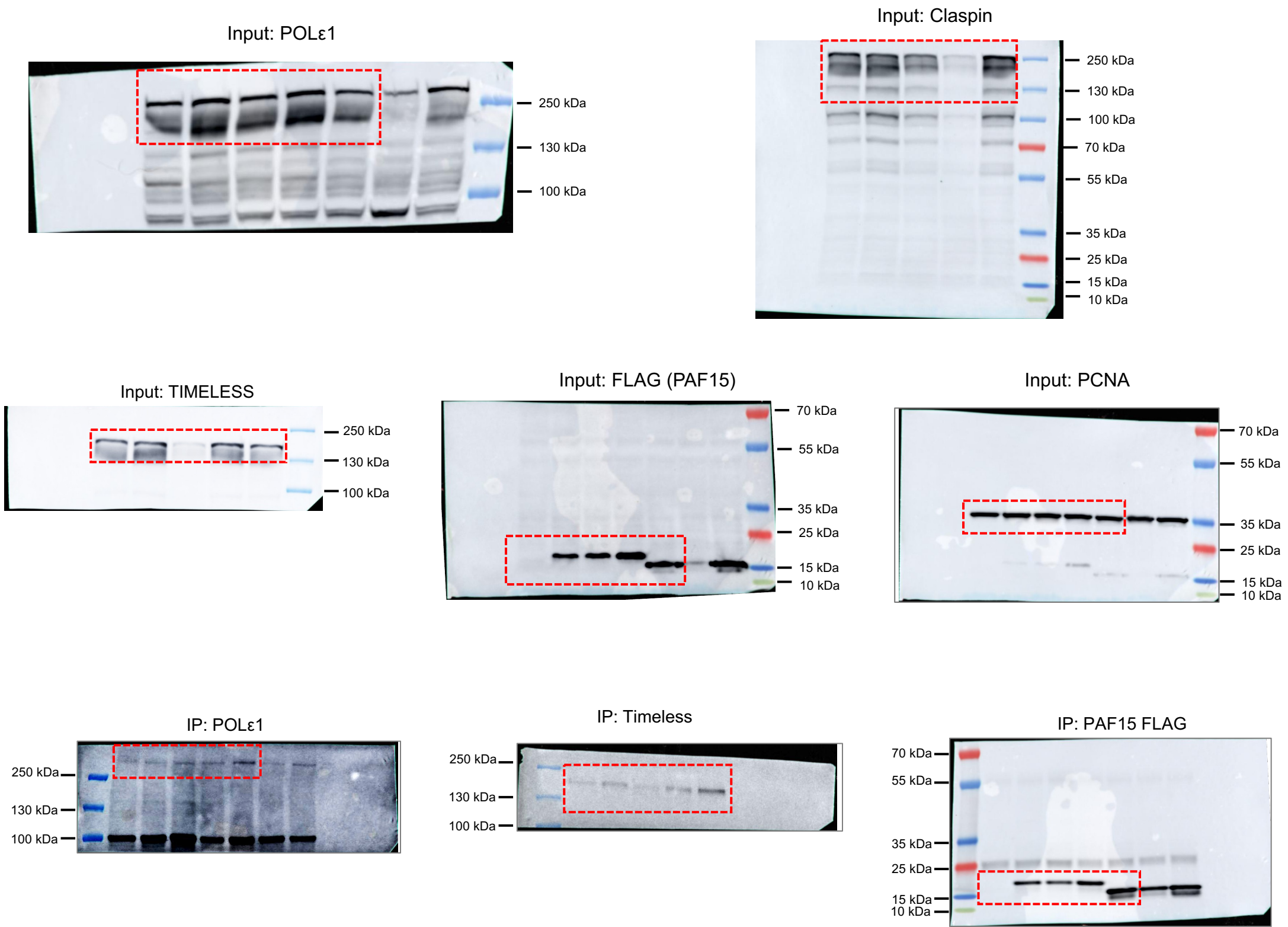
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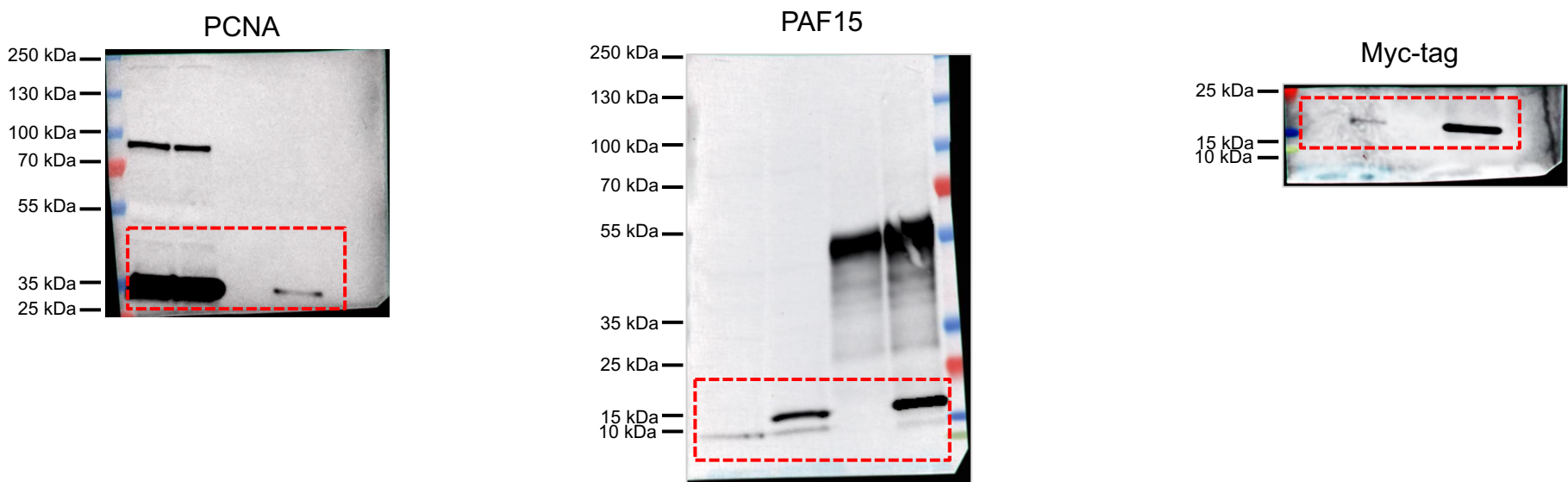
SI Fig 8q: Uncropped immunoblot from Extended Data Fig. 8d



SI Fig 8r: Uncropped immunoblot from Extended Data Fig. 9e

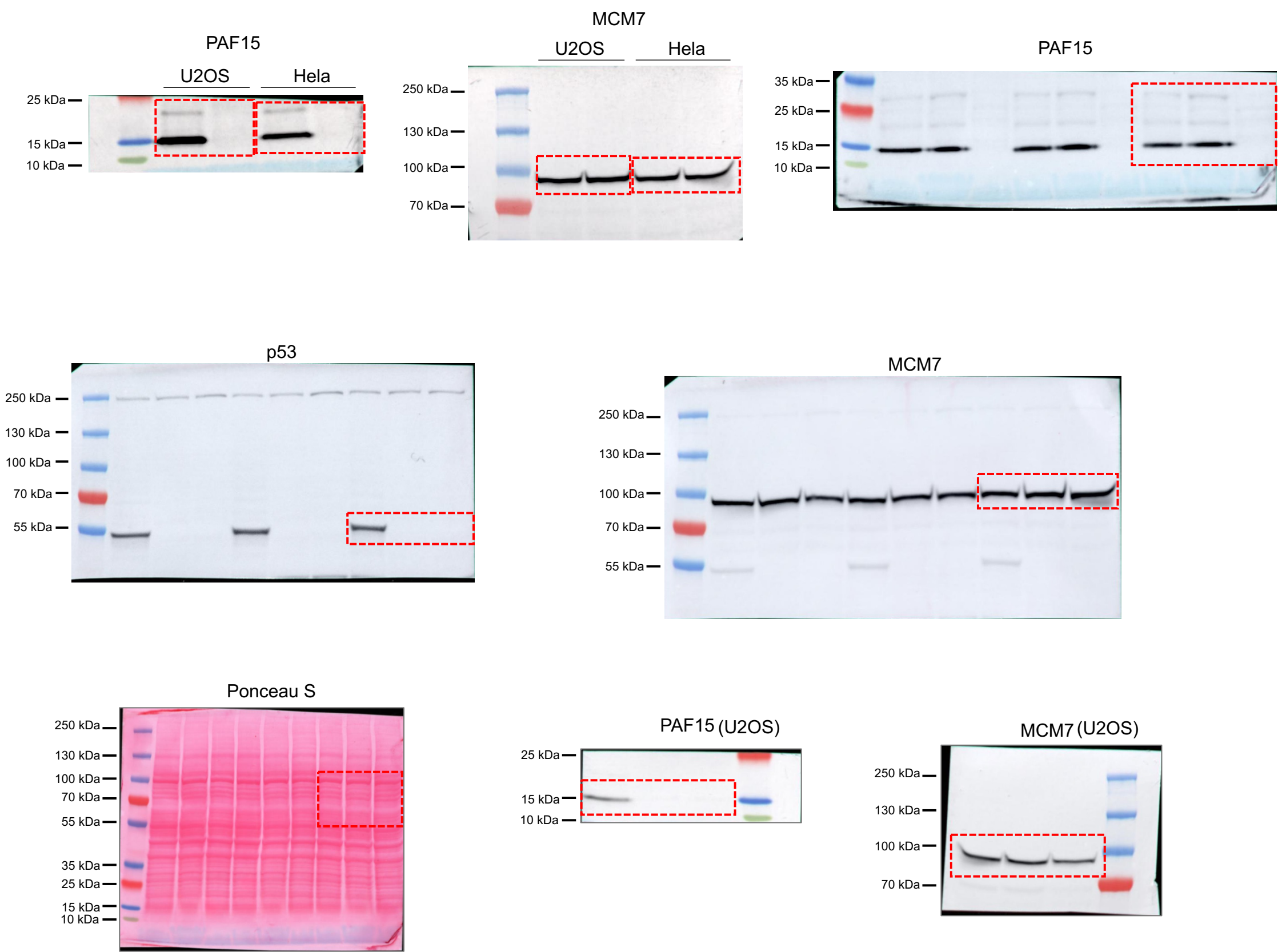


SI Fig 8s: Uncropped immnoblot from SI Figure. 3b

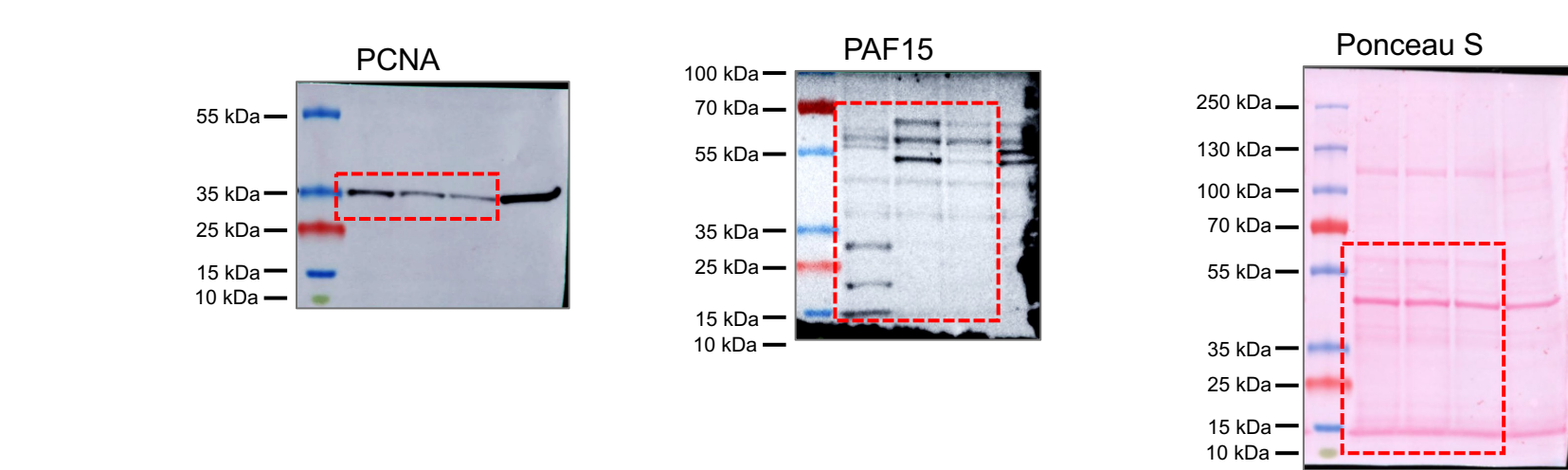




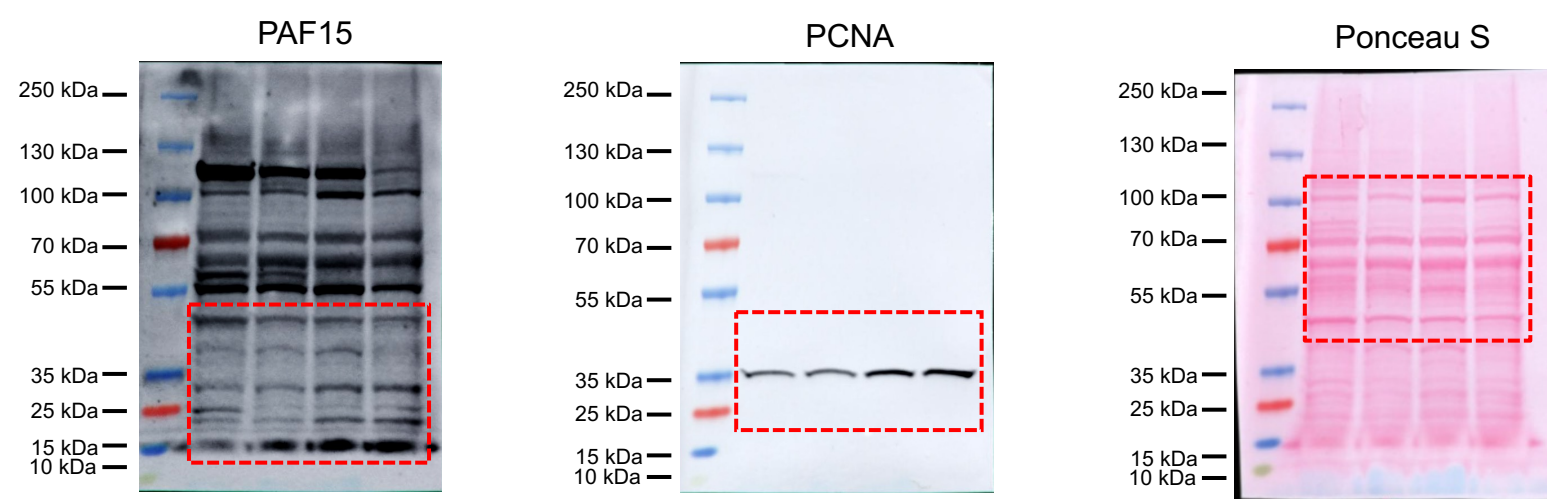
SI Fig 8t: Uncropped immunoblot from SI Figure. 5a



SI Fig 8u: Uncropped immunoblot from SI Figure. 5b



SI Fig 8v: Uncropped immunoblot from SI Figure. 7a



SI Figure 8: Uncropped immunoblot source data from main figures, extended dat figures and supplementary information figures. **a**, uncropped immunoblot from Fig. 1h. **b**, uncropped immunoblot from Fig. 2h. **c**, uncropped immunoblot from Fig. 3a. **d**, uncropped immunoblot from Fig. 4c. **e**, uncropped immunoblot from Fig. 4h. **f**, uncropped immunoblot from Fig. 5j. **g**, uncropped immunoblot from Extended Data Fig. 1h. **h**, uncropped immunoblot from Extended Data Fig. 1i. **i**, uncropped immunoblot from Extended Data Fig. 2f. **j**, uncropped immunoblot from Extended Data Fig. 3i. **k**, uncropped immunoblot from Extended Data Fig. 4a. **l**, uncropped immunoblot from Extended Data Fig. 5a. **m**, uncropped immunoblot from Extended Data Fig. 5f. **n**, uncropped immunoblot from Extended Data Fig. 6i. **o**, uncropped immunoblot from Extended Data Fig. 7g. **p**, uncropped immunoblot from Extended Data Fig. 7h. **q**, uncropped immunoblot from Extended Data Fig. 8d. **r**, uncropped immunoblot from Extended Data Fig. 9e. **s**, uncropped immunoblot from SI Figure. 3b. **t**, uncropped immunoblot from SI Figure. 5a. **u**, uncropped immunoblot from SI Figure. 5b. **v**, uncropped immunoblot from SI Figure. 7a.